

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

JOINT INVENTORS

"EXPRESS MAIL" mailing label No.
EK657824791US.

Date of Deposit: March 16, 2001

I hereby certify that this paper (or fee) is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 CFR §1.10 on the date indicated above and is addressed to: Commissioner for Patents, Washington, D.C. 20231


Richard Zimmermann

**APPLICATION FOR
UNITED STATES LETTERS PATENT**

S P E C I F I C A T I O N

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Eugene Medlock a citizen of United States, residing at 3974 Oak Hollow Circle, Westlake Village, California 91302 and Richard Yeh a citizen of United States, residing at 2250 North Tripphammer Road, P1C, Ithaca, New York 14850 and Scott M. Silbiger a citizen of United States, residing at 21520 Burbank Blvd., #301, Woodland Hills, California 91367 and Gary S. Elliot a citizen of United States, residing at 324 Greenmoor Place, Thousand Oaks, California 91361 and Hung Q. Nguyen a citizen of United States, residing at 881 St. Charles Drive, Apt. 10, Thousand Oaks, California 91360 and Shuqian Jing a citizen of United States, residing at 3254 Bordero Lane, Thousand Oaks, California 91362 hav invented a new and useful IL-17 RECEPTOR LIKE MOLECULES AND USES THEREOF, of which the following is a specification.

IL-17 RECEPTOR LIKE MOLECULES AND USES THEREOF

Related Application

7/23/01

This application claims priority from U.S. Patent Application serial no. 09/723,232 (attorney docket no. 01017/36917) filed November 27, 2000 which claims priority from U.S. provisional patent application serial no. 60/189,923 filed March 16, 2000 (attorney docket no. A-666-P) and U.S. provisional application serial no. 60/204,208 (attorney docket no. A-666A-P) filed May 12, 2000. This application also claims priority from U.S. provisional application no. 60/266,159 (attorney docket no. 01017/37128) filed February 2, 2001 which claims benefit of U.S. provisional application no. 60/213,125 (attorney docket no. A-707-P) filed June 22, 2000. All of the above-identified applications are incorporated herein by reference in their entirety.

Field of the Invention

The present invention relates to novel IL-17 receptor like polypeptides and nucleic acid molecules encoding the same. The invention also relates to vectors, host cells, pharmaceutical compositions, selective binding agents and methods for producing IL-17 receptor like polypeptides. Also provided for are methods for the diagnosis, treatment, amelioration, and/or prevention of diseases associated with IL-17 receptor like polypeptides.

Background of the Invention

Technical advances in the identification, cloning, expression and manipulation of nucleic acid molecules have greatly accelerated the discovery of novel therapeutics based upon deciphering the human genome. Rapid nucleic acid sequencing techniques can now generate sequence information at

unprecedented rates and, coupled with computational analyses, allow the assembly of overlapping sequences into partial and entire genomes and the identification of polypeptide-encoding regions. A comparison of a predicted amino acid sequence
5 against a database compilation of known amino acid sequences can allow one to determine the extent of homology to previously identified sequences and/or structural landmarks. The cloning and expression of a polypeptide-encoding region of a nucleic acid molecule provides a polypeptide product for
10 structural and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides to create variants and derivatives thereof may confer advantageous properties on a product for use as a therapeutic.

In spite of the significant technical advances in genome
15 research over the past decade, the potential for the development of novel therapeutics based on the human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics, or those encoding polypeptides, which may act as "targets" for therapeutic
20 molecules, have still not been identified. In addition, structural and functional analyses of polypeptide products from many genes have not been undertaken.

Accordingly, it is an object of the invention to identify novel polypeptides and nucleic acid molecules encoding the
25 same, which have diagnostic or therapeutic benefit.

Summary of the Invention

The present invention relates to novel IL-17 receptor like nucleic acid molecules and encoded polypeptides.

30 The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof;

5 (b) a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

10 (c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the polypeptide encoded by the nucleotide sequence has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof; and

15 (d) a nucleotide sequence complementary to any of (a)-(c).

The invention also provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

20 (a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

30 (b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof, wherein the encoded polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(c) a nucleotide sequence of any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof; (a), or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(d) a nucleotide sequence of any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof; or (a)-(d) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d), wherein the polypeptide encoded by the nucleotide sequence has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof; and

(f) a nucleotide sequence complementary to any of (a)-(e).

The invention further provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(b) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, with at least one amino acid insertion, wherein the polypeptide has an activity of the

polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(c) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(d) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(e) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(f) a nucleotide sequence of (a)-(e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the polypeptide encoded by the nucleotide sequence has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof; and

(h) a nucleotide sequence complementary to any of (a)-(e).

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group
5 consisting of:

(a) an amino acid sequence for an ortholog of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, wherein the encoded polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID
10 NO:5, or SEQ ID NO:7, including combinations thereof;

(b) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, wherein the polypeptide
15 has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(c) a fragment of the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including
20 combinations thereof, comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(d) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth
25 in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or at least one of (a)-(b) wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including
30 combinations thereof.

The invention further provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

5 (a) the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

10 (b) the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including
15 combinations thereof;

(c) the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in
20 any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof;

(d) the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, which has a C- and/or N-terminal truncation, wherein
25 the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof; and

(e) the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations
30 thereof, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and

N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

Also provided are fusion polypeptides comprising the amino acid sequences of (a)-(e) above.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein, recombinant host cells comprising recombinant nucleic acid molecules as set forth herein, and a method of producing an IL-17 receptor like polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced. These expression vectors include baculovirus expression vectors which utilize insect cells for expression.

The host cells of the present invention also include those comprising a IL-17 receptor nucleic acid molecule operatively linked to a regulatory sequence other than the promoter of the native IL-17 receptor like polypeptide. These host cells also include those modified by transformation or transfection with a heterologous nucleic acid, including promoters and transcription factors, that promotes transcription or translation of the nucleic acid comprising the sequence of SEQ ID NO: 1, 4, or 6 or a allelic variant or fragment thereof.

Vectors containing the cDNA inserts corresponding to SEQ ID NOS: 1, 4 and 6 (denoted HIL-17RB-1, HIL-17RB-2 and HIL17RB-3, respectively) have been deposited on March 14, 2001 with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110, U.S.A. under Accession Nos. _____, _____ and _____, respectively). Included in the present invention are isolated polynucleotides comprising the protein

coding or mature protein coding regions of the respective cDNA inserts, as well as mature protein or extracellular domains thereof obtainable by expressing cDNA in suitable host cells.

5 A transgenic non-human animal comprising a nucleic acid molecule encoding an IL-17 receptor like polypeptide is also encompassed by the invention. The IL-17 receptor like nucleic acid molecules are introduced into the animal in a manner that allows expression and increased levels of the IL-17 receptor like polypeptide, which may include increased circulating
10 levels. The transgenic non-human animal is preferably a mammal. Also provided is a transgenic non-human animal comprising a disruption in the nucleic acid molecule encoding IL-17 receptor like polypeptide, which will knock out or significantly decrease expression if the IL-17 receptor like
15 polypeptide.

Also provided are derivatives of the IL-17 receptor like polypeptides of the present invention.

20 Analogs of the IL-17 receptor like polypeptides are provided for in the present invention which result from conservative and/or non-conservative amino acids substitutions of the IL-17 receptor like polypeptides of SEQ ID NO: 2, 5 or 7. Such analogs include an IL-17 receptor like polypeptide wherein, for example the amino acid at position 167 of SEQ ID NO: 2, position 225 of SEQ ID NO: 5 or position 50 of SEQ ID
25 No: 7 is methionine, leucine, isoleucine, or phenylalanine; the amino acid at position 261 of SEQ ID NO: 2, position 319 of SEQ ID NO: 5 or position 144 of SEQ ID NO: 7 is cysteine, serine or alanine; the amino acid at position 299 of SEQ ID NO: 2, position 357 of SEQ ID NO: 5 or position 212 of SEQ ID
30 NO: 7 is leucine, norleucine, glutamine, asparagine, arganine, or 1,4, diamino-butyric Acid; the amino acid at position 313 of SEQ ID NO: 2, position 371 of SEQ ID NO: 5 or position 193

of SEQ ID NO: 7 is tryptophan, tyrosine or phenylalanine; the amino acid at position 413 of SEQ ID NO: 2, position 471 of SEQ ID NO: 5, or position 296 of SEQ ID NO: 7 is glycine, proline or alanine; or the amino acid at position 433 of SEQ ID NO: 2, position 491 of SEQ ID NO: 5 or position 313 of SEQ ID NO: 7 is aspartic acid or glutamic acid.

Additionally provided are selective binding agents such as antibodies and peptides capable of specifically binding the IL-17 receptor like polypeptides of the invention. Such antibodies, polypeptides and small molecules may be agonistic or antagonistic. Antagonistic selective binding agents include those which inhibit binding of a IL-17 receptor like polypeptide to an IL-17E ligand (such as the mature protein amino acid sequence of SEQ ID NO: 23).

Pharmaceutical compositions comprising the nucleotides, polypeptides, or selective binding agents of the present invention and one or more pharmaceutically acceptable formulation agents are also encompassed by the invention. The pharmaceutical compositions are used to provide therapeutically effective amounts of the nucleotides or polypeptides of the present invention. The invention is also directed to methods of using the polypeptides, nucleic acid molecules, and selective binding agents.

The IL-17 receptor like polypeptides and nucleic acid molecules of the present invention may be used to treat, prevent, ameliorate, diagnosis and/or detect diseases and disorders, including those recited herein. Expression analysis in biological, cellular or tissue samples suggests that IL-17 receptor like polypeptide may play a role in the diagnosis and/or treatment of the pathological conditions described herein. This expression can be detected with a diagnostic agent such as a IL-17 receptor like polynucleotide.

The invention encompasses diagnosing a pathological condition or the susceptibility to a pathological condition in a subject caused by or resulting from abnormal (i.e. increased or decreased) levels of IL-17 receptor like polypeptide comprising determining the presence or amount of expression of the IL-17 receptor like polypeptide in a sample and comprising the level of said polypeptide in a biological, tissue or cellular sample from either normal subjects or the subject at an earlier time, wherein susceptibility to a pathological condition is based on the presence or amount of expression of the polypeptide

The present invention also provides a method of assaying test molecules to identify a test molecule which binds to an IL-17 receptor like polypeptide. The method comprises contacting an IL-17 receptor like polypeptide with a test molecule and determining the extent of binding of the test molecule to the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists (candidate inhibitors and stimulators) of an IL-17 receptor like polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of IL-17 receptor like polypeptide or on the activity of IL-17 receptor like polypeptide.

The present invention provides for methods of identifying antagonists or agonists of IL-17 receptor like biological activity comprising contacting a small molecule compound with IL-17 receptor like polypeptides and measuring IL-17 receptor like biological activity in the presence and absence of these small molecules. These small molecules can be a naturally occurring medicinal compound or derived from combinational chemical libraries. In certain embodiments, an IL-17 receptor like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecule which

interacts with an IL-17 receptor like polypeptide to regulate its activity or inhibit ligand binding.

The IL-17 receptor like polypeptide can be used for identifying ligands thereof. Various forms of "expression cloning" have been used for cloning ligands for receptors. See e.g., Davis et al., *Cell*, 87:1161-1169 (1996). These and other IL-17 receptor like ligand cloning experiments are described in greater detail herein. Isolation of the IL-17 receptor like ligand(s) allows for the identification or development of novel agonists and/or antagonists of the IL-17 receptor like signaling pathway.

One ligand (denoted herein as IL-17E) has been identified herein in Example 8. Its nucleotide and amino acid sequences are set forth in SEQ ID NOS: 22 and 23, respectively. The cDNA encodes an open reading frame of 161 amino acids with a predicted signal peptide of 16 amino acids and a predicted mature protein of 145 amino acids. Tissue expression data, homology to other IL-17 ligands and phenotypes of transgenic mice overexpressing IL-17E suggest that this IL-17E ligand (and thus the IL-17 receptor like polypeptides of the present invention which bind to IL-17E) play a role in inflammation, including autoimmune diseases, and in myelopoiesis, particularly in the development, stimulation and/or recruitment of eosinophils and lymphocytes (especially B-lymphocytes). See U.S. provisional patent application serial no. 60/266,159 (attorney docket no. 01017/37128) incorporated herein by reference in its entirety, wherein a IL-17E polypeptide was identified to be the ligand for the IL-17 receptor like polypeptides IL-17RB-2 and IL-17RB-3 (SEQ ID NOS: 2 and 5) of the present invention.

One embodiment of the invention provides for methods of identifying inhibitors of an interaction of an IL-17 receptor

polypeptide with an IL-17E ligand. These methods comprise the steps of detecting binding of an IL-17 receptor like polypeptide (such as polypeptides comprising the mature protein sequence set out in SEQ ID NOS: 2, 5 or 7 or fragments, analogs or variants thereof that retain ligand-binding activity) to IL-17E ligand (such as a polypeptide comprising the mature protein sequence of SEQ ID NO: 23 or fragments, analogs or variants thereof that retain receptor-binding activity), in the presence and absence of a test compound, and identifying the test compound as a candidate inhibitor when the binding is decreased in the presence of the compound. Suitable test compounds include nucleic acid molecules, proteins, peptides, carbohydrates, lipids, organic and inorganic compounds, libraries of which can be screened using known high throughput screening procedures.

The present invention further provides for methods of treating, preventing or ameliorating a pathological condition mediated by IL-17E comprising administering a therapeutically effective amount of a molecule which specifically binds to either IL-17E ligand or IL-17 receptor like polypeptides of the present invention. The invention also provides for a method of inhibiting undesirable interaction of IL-17 receptor like polypeptide with IL-17E ligand comprising administering a therapeutically effective amount of a molecule capable of binding the IL-17 receptor like polypeptide or IL-17E ligand, or a molecule otherwise capable of inhibiting the interaction between IL-17 receptor-like polypeptide with IL-17E ligand. Candidate inhibitors include selective binding agents (including antibodies or derivatives thereof) that are specific for either IL-17E ligand or IL-17 receptor like polypeptides; analogs, fragments or variants of IL-17 receptor like polypeptides of the present invention (e.g. that retain ligand-binding site(s) of the receptor) and fusion proteins

thereof; analogs, fragments or variants of IL-17E ligand (e.g. that retain ability to bind receptor without transducing a signal) and fusion proteins thereof.

Exemplary IL-17E mediated pathological conditions include but are not limited to those conditions related to immune system dysfunction, inflammation (including acute or chronic inflammation), and the progression of cancer. IL-17E polypeptide and polynucleotide may play a role in lymphoma conditions and increased expression of IL-17E polypeptide or polynucleotide may be indicative of a prelymphoma state. Other conditions involving IL-17E include infection.

The invention also provides for a method of inhibiting undesirable interaction of IL-17 receptor like polypeptide with IL-17E ligand comprising administering a therapeutically effective amount of a molecule capable of binding the IL-17 receptor like polypeptide or IL-17E ligand.

Methods of regulating expression and modulating (i.e., increasing or decreasing) levels or activity of an IL-17 receptor like polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule encoding an IL-17 receptor like polypeptide. In another method, a nucleic acid molecule comprising elements that will regulate or modulate the expression of an IL-17 receptor like polypeptide may be administered. Conversely, selective binding agents or antisense oligonucleotides may be administered to treat, prevent or ameliorate pathological conditions related to increased levels or activity of IL-17 receptor like polypeptide. Examples of these methods include gene therapy, cell therapy, and anti-sense therapy as further described herein.

Yeast two-hybrid screens have been extensively used to identify and clone receptors for protein ligands. (Chien et al., Proc. Natl. Acad. Sci. U.S.A., 88: 9578-9583, 1991). The isolation of a IL-17 receptor like polypeptide binding partner is useful for identifying or developing novel agonists and antagonists of the IL-17 receptor like polypeptide activity. Such agonists and antagonists include but are not limited to soluble anti-IL-17 receptor like polypeptides (e.g. fragments lacking all or part of the transmembrane and/or cytoplasmic region(s) or fragments of the extracellular region(s) that retain ligand binding activity, analogs or variants thereof, and fusions thereof to heterologous polypeptides such as constant domains of an immunoglobulin or fragments or variants thereof that retain the ability to prolong half-life in circulation), IL-17 receptor like selective binding agents (such as antibodies and derivatives thereof including chimeric, humanized or human antibodies or fragments thereof that specifically bind to the IL-17 receptor like polypeptide or its ligand-binding sites), small molecules, peptides or derivatives thereof capable of binding IL-17 receptor like polypeptide or its ligand binding site(s) or antisense oligonucleotides (e.g., that specifically bind to IL-17 receptor like encoding DNA or RNA or regulatory sequences and inhibit expression of IL-17 like receptor like polypeptide), any of which can be used for potentially treating one or more diseases or disorders disclosed, including those recited herein.

The invention further encompasses methods for determine the presence of IL-17 receptor like nucleic acids in a biological, tissue or cellular sample . These methods comprise the steps of providing a biological sample suspected of containing IL-17 receptor like nucleic acids; contacting the biological sample with a diagnostic reagent of the present invention under conditions wherein the diagnostic reagent will

hybridize with IL-17 receptor like nucleic acids contained in said biological sample; detecting hybridization between nucleic acid in the biological sample and the diagnostic reagent; and comparing the level of hybridization between the biological sample and diagnostic reagent with the level of hybridization between a known concentration of IL-17 receptor like nucleic acid and the diagnostic reagent. The polynucleotide detected in these methods may be an IL-17 receptor like DNA or and IL-17 receptor like RNA.

The invention also provides for a device which comprises a membrane suitable for implantation in a patient; and cells encapsulated within said membrane, wherein said cells secrete an IL-17 receptor like polypeptide of the invention wherein the membrane is permeable to the protein product and impermeable to materials detrimental to said cells. The invention further provides for a device which comprises a membrane suitable for implantation and the IL-17 receptor like polypeptide encapsulated in a membrane that is permeable to the polypeptide

The present invention also encompasses diagnostic reagents, including detectably labeled polynucleotides encoding the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 5 or SEQ ID NO: 7, fragments, variants, homologs thereof. Further, the invention provides for methods of determining the presence of IL-17 receptor like nucleic acids (including DNA and RNA) in biological, cellular and tissue samples by contacting said sample with a diagnostic reagent as described herein that will hybridize with IL-17 receptor like nucleic acid contained in said sample, detecting said hybridization and comparing the level of hybridization between the sample and diagnostic reagent with a the level of hybridization between a known concentration of IL-17 receptor like nucleic acid and the diagnostic reagent.

Brief Description of the Figures

Fig 1
Figure 1 depicts a nucleic acid sequence (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of a first human IL-17 receptor like polypeptide.

Figure 2 depicts homology of a first human IL-17 receptor like polypeptide amino acid sequence (SEQ ID NO:2) and a known IL-17 receptor family member (SEQ ID NO:3).

Fig 2
Figure 3 depicts a nucleic acid sequence (SEQ ID NO:4) and amino acid sequence (SEQ ID NO:5) of a second human IL-17 receptor like polypeptide.

Figure 4 depicts homology of a second human IL-17 receptor like polypeptide amino acid sequence (SEQ ID NO:5) and a known IL-17 receptor family member (SEQ ID NO:3).

Fig 3
Figure 5 depicts a nucleic acid sequence (SEQ ID NO:6) and amino acid sequence (SEQ ID NO:7) of a third human IL-17 receptor like polypeptide.

Figure 6 depicts homology of a third human IL-17 receptor like polypeptide amino acid sequence (SEQ ID NO:7) and a known IL-17 receptor family member (SEQ ID NO:3).

Figure 7 depicts an overlap of amino acid sequences of the first (SEQ ID NO: 2; IL-17RB-2), second (SEQ ID NO: 5; IL-17RB-3), and third (SEQ ID NO: 7) human IL-17 receptor like polypeptides. The underlined sequence is the predicted transmembrane domain which spans residues 293 to 313 of SEQ ID NO: 2, residues 351 to 371 of SEQ ID NO: 5 and residues 176 to 196 of SEQ ID NO: 7. The predicted signal peptide is in bold which spans residues 14 of SEQ ID NOS: 2 and 5. Therefore the predicted extra-cellular sequence spans amino acids 14 to 292 of SEQ ID NO: 2 and amino acids 14 to 350 of SEQ ID NO: 5.

Figure 8 depicts a Northern blot detecting expression of the IL-17 like overexpressing transgene in necropsied transgenic founder mice (nos. 1, 16, 27, 29, 55, 61, 20, 52, and 66). The control mice (nos. 2, 17, 53 and 65) are non-transgenic littermates. The lane marked "bl" is a blank lane and the positive control (+) was the IL-17 like cDNA. The presence of a 0.54 kb band is indicative of transgene expression.

Figure 9 depicts a Northern blot detecting expression of the IL-17 like overexpressing transgene in hepatectomized transgenic founder mice (nos. 10, 11, 30, 31, 33, 37, 46, 67, and 68). The control mice (nos. 32, 35, 36 and 45) are non-transgenic littermates. The lane marked "MI" represents the microinjection fragment which was loaded as a positive control. The presence of a 0.54 kb band is indicative of transgene expression.

Figure 10 depicts hematoxylin and eosin (A,B, G-J), B220 (C,D) and F4/80 (E,F) stained sections of lymph node (A-H) or bone marrow (I,J) from IL-17 like transgenic mice (B,D,F,H) or non-transgenic control mice (A,C,E,G). Panels A-F illustrate that the IL-17 like transgenic lymph node was markedly enlarged with its normal architecture disrupted due to a marked cellular infiltrate (asterisk in panel B) that contained large numbers of B220 positive B lymphocytes cells (panel D) and some F4/80 staining macrophages. Panel H illustrates that this cellular infiltrate also contained numerous eosinophils (arrowheads) as well as multinucleated inflammatory giant cells (arrows).

Figure 11 depicts hematoxylin and eosin (A,B; E-I) and B220 (C,D) stained sections of lymph bone marrow (A,B), spleen (C-F) and kidney (G-J) from IL-17 like transgenic mice (B,D,F,H,J) or non-transgenic control mice (A,C,E,G,I). Panel A illustrates marked eosinophilic myeloid hyperplasia. Panel

D illustrates lymphoid hyperplasia with a predominance of B220 positive B cells (arrows) in the IL-17 like transgenic mouse spleen, while panel F illustrates eosinophilic myeloid hyperplasia in the IL-17 like transgenic splenic red pulp compared to the non-transgenic splenic red pulp (E). Panels H and J illustrate renal pelvic dilation (arrow in H) with a marked eosinophilic inflammatory infiltration in the renal pelvis (pyelonephritis, panel J).

Figure 12 depicts a bar chart histogram showing a significant increase in absolute numbers of CD19+ B lymphocytes in the peripheral blood of 4 out of 9 IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

Figure 13 depicts a bar chart histogram showing an increase in absolute numbers of CD19+ B lymphocytes in the spleens of 5 out of 10 IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

Figure 14 depicts a bar chart histogram showing a slight decrease in absolute numbers of CD19+ B lymphocytes in the bone marrow of IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

Figure 15 depicts a bar chart histogram showing an increase in absolute numbers of CD4+ T lymphocytes in the peripheral blood of 4 out 9 IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

Figure 16 depicts a bar chart histogram showing an increase in absolute numbers of CD4+ T lymphocytes in the spleens of IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

Figure 17 depicts scatter plots representative of the changes occurring in the IL-17 like transgenic mice vs. their non-transgenic littermate controls. The two top plots labeled

"A" are 2-color flow cytometric dot plots where CD45R+ and IL-17 like-Fc labeling are being depicted on their respective axes. Control plot "A" shows an absence of CD45R+/IL-17 like-Fc+ cells in the region R1 whereas in the transgenic plot "A", this population was present in region R1 and represented 8% of the total granulocyte population. In the corresponding Forward vs. Side scatter plot ("B" and "C") these cells are depicted as pink colored dots. This population was absent in the control plot "B".

Figure 18 depicts scatter plots representative of the changes occurring in the IL-17 like transgenic mice vs. their non-transgenic littermate controls. The two top plots labeled "A" are 2-color flow cytometric dot plots where CD4 and IL-17 like-Fc labeling are being depicted on their respective axis. Control plot "A" shows an absence of CD4+/IL-17 like-Fc+ cells in the region R1, whereas in the transgenic plot "A", this population was present in region R1 and represented 14% of the total granulocyte population. In the corresponding Forward vs. Side scatter plots (size vs. granularity), the IL-17 like transgenic mice (B) these cells are located just above the region where granulocytes are typically found (red colored dots). These cells are absent in the control plot "B". Furthermore, for the transgenic mice (A), there is an emergence of a population of cells that was neither CD4+ nor IL-17 like-Fc+ (region R2) but that has the scatter properties of eosinophils, localizing to the left of the granulocytes in the Forward vs. Side scatter plot "B" (green colored dots). This population was absent in the control plot "B".

Figure 19 depicts a bar chart histogram showing an increase in absolute numbers of rhIL-17 like-Fc+/CD45R+ granulocyte-like cells in the bone marrow of 5 out of 10 IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

Figure 20 depicts a bar chart histogram showing an

increase in absolute numbers of rhIL-17 like-Fc+/CD4+ granulocyte-like cells in the bone marrow of IL-17 like transgenic mice as compared to the non-transgenic littermate controls.

5 Figure 21 depicts an example of a typical Forward vs. Side scatter plot (size vs. granularity). Cells in the gate can be sorted to give a purified population.

Figure 22 sets out the sequence of the IL-17RB-2 fusion protein (SEQ ID NO: 24) comprising the extra-cellular domain
10 of IL-17-RB-2 (SEQ ID NO: 2) and the FC fusion peptide (SEQ ID NO: 21). The FC fusion portion of the amino acid sequence is underlined and the native signal peptide of IL-17RB-2 is bold.

Figure 23 sets out the sequence of the IL-17RB-3 fusion protein (SEQ ID NO: 25) comprising the extra-cellular domain
15 of IL-17-RB-3 (SEQ ID NO: 5) and the FC fusion peptide (SEQ ID NO: 21). The FC fusion portion of the amino acid sequence is underlined and the native signal peptide of IL-17RB-3 is bold.

Detailed Description of the Invention

20 The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein.

Definitions

The terms "IL-17 receptor like gene" or "IL-17 receptor like nucleic acid molecule" or "polynucleotide" refers to a nucleic acid molecule comprising or consisting of a nucleotide sequence as set forth in any of SEQ ID NO:1, SEQ ID NO:4, or
30 SEQ ID NO:6, including combinations thereof; a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations

thereof (such as, but not limited to fusion proteins as described herein); the nucleotide sequences of the DNA insert(s) in Amgen deposit no. A-666A-P (hIL-17rl.1, hIL-17rl.2, and hIL-17rl.3); and nucleic acid molecules as defined
5 herein.

The term "IL-17 receptor like polypeptide" refers to a polypeptide comprising the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, OR SEQ ID NO:7, including combinations thereof, and related polypeptides. Related polypeptides
10 include: IL-17 receptor like polypeptide allelic variants, IL-17 receptor like polypeptide orthologs, IL-17 receptor like polypeptide splice variants, IL-17 receptor like polypeptide variants and IL-17 receptor like polypeptide derivatives. IL-17 receptor like polypeptides may be mature polypeptides, as
15 defined herein, and may or may not have an amino terminal methionine residue, depending on the method by which they are prepared.

The term "IL-17 receptor like polypeptide allelic variant" refers to one of several possible naturally occurring
20 alternate forms of a gene occupying a given locus on a chromosome of an organism or a population of organisms.

The term "IL-17 receptor like polypeptide derivatives" refers to the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, OR SEQ ID NO:7, including combinations thereof,
25 IL-17 receptor like polypeptide allelic variants, IL-17 receptor like polypeptide orthologs, IL-17 receptor like polypeptide splice variants, or IL-17 receptor like polypeptide variants, as defined herein, that have been chemically modified.

The term "IL-17 receptor like polypeptide fragment" refers to a polypeptide that comprises a truncation at the
30

amino terminus (with or without a leader sequence) and/or a truncation at the carboxy terminus of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, OR SEQ ID NO:7, including combinations thereof, IL-17 receptor like polypeptide allelic variants, IL-17 receptor like polypeptide orthologs, IL-17 receptor like polypeptide splice variants and/or an IL-17 receptor like polypeptide variant having one or more amino acid additions or substitutions or internal deletions (wherein the resulting polypeptide is at least 6 amino acids or more in length) as compared to the IL-17 receptor like polypeptide amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, OR SEQ ID NO:7, including combinations thereof. IL-17 receptor like polypeptide fragments may result from alternative RNA splicing or from *in vivo* protease activity. For transmembrane or membrane-bound forms of an IL-17 receptor like polypeptide, preferred fragments include soluble forms such as those lacking a transmembrane or membrane-binding domain. For example, a soluble fragment of the IL-17 receptor like polypeptide is a polypeptide comprising SEQ ID NO: 2 which lacks amino acids 293 to 313 or a polypeptide comprising SEQ ID NO: 5 which lacks nucleotides 351 to 371. Preferred IL-17 receptor like polypeptide fragments retain the ability to bind to IL-17 receptor like polypeptide ligands such as IL-17E of SEQ ID NO: 23. In preferred embodiments, truncations comprise about 10 amino acids, or about 20 amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or more than about 100 amino acids. The polypeptide fragments so produced will comprise about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 150 amino acids, or about 200 amino acids. Such IL-17 receptor like polypeptide fragments may optionally comprise an amino terminal methionine residue. It will be appreciated that such fragments can be used, for

example, to generate antibodies to IL-17 receptor like polypeptides.

The term "IL-17 receptor like fusion polypeptide" refers to a fusion of one or more amino acids (such as a heterologous peptide or polypeptide) at the amino or carboxy terminus of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, IL-17 receptor like polypeptide allelic variants, IL-17 receptor like polypeptide orthologs, IL-17 receptor like polypeptide splice variants, or IL-17 receptor like polypeptide variants having one or more amino acid deletions, substitutions or internal additions as compared to the IL-17 receptor like polypeptide amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof. It will be appreciated that fusion proteins include combinations of polypeptide amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7.

The term "IL-17 receptor like polypeptide ortholog" refers to a polypeptide from another species that corresponds to IL-17 receptor like polypeptide amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof. For example, mouse and human IL-17 receptor like polypeptides are considered orthologs of each other.

The term "IL-17 receptor like polypeptide splice variant" refers to a nucleic acid molecule, usually RNA, which is generated by alternative processing of intron sequences in an RNA transcript of IL-17 receptor like polypeptide amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

5 The term "IL-17 receptor like polypeptide variants" refers to IL-17 receptor like polypeptides comprising amino acid sequences having one or more amino acid sequence substitutions, deletions (such as internal deletions and/or
10 IL-17 receptor like polypeptide fragments), and/or additions (such as internal additions and/or IL-17 receptor like fusion polypeptides) as compared to the IL-17 receptor like polypeptide amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, OR SEQ ID NO:7, including combinations thereof (with or without a leader sequence). Variants may be naturally occurring (e.g., IL-17 receptor like polypeptide allelic variants, IL-17 receptor like polypeptide orthologs and IL-17 receptor like polypeptide splice variants) or artificially constructed. Such IL-17 receptor like
15 polypeptide variants may be prepared from the corresponding nucleic acid molecules having a DNA sequence that varies accordingly from the DNA sequence as set forth in any of SEQ ID NO:1, SEQ ID NO:4, OR SEQ ID NO:6, including combinations thereof. In preferred embodiments, the variants have from 1
20 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, wherein the substitutions may be conservative, or non-conservative, or
25 any combination thereof.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an
30 epitope of that antigen. An antigen may have one or more epitopes.

The term specific binding reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens.

The term "biologically active IL-17 receptor like polypeptides" refers to IL-17 receptor like polypeptides having at least one activity characteristic of the polypeptide comprising the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

The terms "effective amount" and "therapeutically effective amount" each refer to the amount of a IL-17 receptor like polypeptide or IL-17 receptor like nucleic acid molecule used to support an observable level of one or more biological activities of the IL-17 receptor like polypeptides as set forth herein.

The term "expression vector" refers to a vector which is suitable for use in a host cell and contains nucleic acid sequences which direct and/or control the expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

100-40997-034604

The term "identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms").

The term "similarity" is a related concept, but in contrast to "identity", refers to a measure of similarity which includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the per cent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the invention that (1) has been separated from at least about 50 percent of proteins, lipids, carbohydrates or other materials with which it is naturally found when total DNA is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3)

is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from at least one contaminating nucleic acid molecule with which it is naturally associated. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates or other materials with which it is naturally found when isolated from the cell source, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked to in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "mature IL-17 receptor like polypeptide" refers to an IL-17 receptor like polypeptide lacking a leader sequence. A mature IL-17 receptor like polypeptide may also include other modifications such as proteolytic processing of the amino terminus (with or without a leader sequence) and/or the carboxy terminus, cleavage of a smaller polypeptide from a

larger precursor, N-linked and/or O-linked glycosylation, and the like.

10040927 034504

The term "nucleic acid sequence" or "nucleic acid molecule" refers to a DNA or RNA sequence. The term encompasses molecules formed from any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxy-methylaminomethyluracil, dihydrouracil, inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "naturally occurring" or "native" when used in

connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as
5 used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to
10 perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of
15 directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence
20 and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing
25 or enhancing the delivery of the IL-17 receptor like polypeptide, IL-17 receptor like nucleic acid molecule or IL-17 receptor like selective binding agent as a pharmaceutical composition.

The term "selective binding agent" refers to a molecule
30 or molecules having specificity for an IL-17 receptor like polypeptide. Selective binding agents include antibodies, such as polyclonal antibodies, monoclonal antibodies (mABs),

chimeric antibodies, CDR-grafted antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound forms, as well as fragments, regions, or derivatives thereof which are provided by known techniques, including, but not limited to enzymatic cleavage, peptide synthesis, or recombinant techniques.

IL-17 receptor like polypeptides, fragments, variants, and derivatives may be used to prepare IL-17 receptor like selective binding agents using methods known in the art. Thus, antibodies and antibody fragments that bind IL-17 receptor like polypeptides are within the scope of the present invention. Antibody fragments include those portions of the antibody which bind to an epitope on the IL-17 receptor like polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions. These antibodies may be, for example, polyclonal monospecific polyclonal, monoclonal, recombinant, chimeric, humanized, human, single chain, and/or bispecific.

As used herein, the terms, "specific" and "specificity" refer to the ability of the selective binding agents to bind to human IL-17 receptor like polypeptides and not to bind to human non-IL-17 receptor like polypeptides. It will be appreciated, however, that the selective binding agents may also bind orthologs of the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, that is, interspecies versions thereof, such as mouse and rat polypeptides.

The term "transduction" is used to refer to the transfer

of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, for example, Graham et al., *Virology*, 52:456 (1973); Sambrook et al., *Molecular Cloning, a laboratory Manual*, Cold Spring Harbor Laboratories (New York, 1989); Davis et al., *Basic Methods in Molecular Biology*, Elsevier, 1986; and Chu et al., *Gene*, 13:197 (1981). Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

Relatedness of Nucleic Acid Molecules
and/or Polypeptides

It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecule of any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof; and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/or a deletion of one or more amino acid residues compared to the polypeptide in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

Fragments include molecules which encode a polypeptide of at least about 25 amino acid residues, or about 50, or about 75, or about 100, or greater than about 100 amino acid residues of the polypeptide of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

In addition, related IL-17 receptor like nucleic acid molecules include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully complementary sequence of the nucleic acid molecule of any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof, or of a molecule encoding a polypeptide, which polypeptide comprises the amino acid sequence as shown in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or of a nucleic acid fragment as defined herein, or of a nucleic acid fragment encoding a polypeptide as defined herein. Hybridization probes may be prepared using the IL-17 receptor like sequences provided herein to screen cDNA, genomic or synthetic DNA libraries for related sequences.

Regions of the DNA and/or amino acid sequence of IL-17 receptor like polypeptide that exhibit significant identity to known sequences are readily determined using sequence alignment algorithms as described herein and those regions may
5 be used to design probes for screening.

The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs.
10 Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.015M sodium chloride, 0.0015M sodium citrate at 65-68°C or 0.015M
15 sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989); Anderson et al., Nucleic Acid Hybridisation: a practical approach, Ch. 4, IRL Press Limited
20 (Oxford, England).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. Other agents may be included in the
25 hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate (NaDodSO₄ or SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA
30 (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed

without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson et al., Nucleic Acid Hybridisation: a Practical Approach, Ch. 4, IRL Press Limited (Oxford, England).

Factors affecting the stability of a DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

$$T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{Na}^+]) + 0.41(\% \text{G+C}) - 600/N - 0.72(\% \text{formamide})$$

where N is the length of the duplex formed, [Na+] is the molar concentration of the sodium ion in the hybridization or washing solution, %G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015M sodium chloride, 0.0015M sodium citrate at 50-65°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 20% formamide at 37-50°C. By way of example, a "moderately stringent" condition of 50°C in 0.015 M sodium ion will allow about a 21% mismatch.

It will be appreciated by those skilled in the art that

there is no absolute distinction between "highly" and "moderately" stringent conditions. For example, at 0.015M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences, one skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1M NaCl* for oligonucleotide probes up to about 20nt is given by:

$$T_m = 2^{\circ}\text{C per A-T base pair} + 4^{\circ}\text{C per G-C base pair}$$

*The sodium ion concentration in 6X salt sodium citrate (SSC) is 1M. See Suggs et al., Developmental Biology Using Purified Genes, p. 683, Brown and Fox (eds.) (1981).

High stringency washing conditions for oligonucleotides are usually at a temperature of 0-5°C below the T_m of the oligonucleotide in 6X SSC, 0.1% SDS.

In another embodiment, related nucleic acid molecules comprise or consist of a nucleotide sequence that is about 70 percent identical to the nucleotide sequence as shown in any of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:6, including combinations thereof, or comprise or consist essentially of a nucleotide sequence encoding a polypeptide that is about 70 percent identical to the polypeptide as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof. In preferred embodiments, the nucleotide sequences are about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequence as shown in any of SEQ ID NO:1, SEQ ID NO:4, or SEQ

ID NO:6, including combinations thereof, or the nucleotide sequences encode a polypeptide that is about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the polypeptide sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

Differences in the nucleic acid sequence may result in conservative and/or non-conservative modifications of the amino acid sequence relative to the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof.

Conservative modifications to the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof (and the corresponding modifications to the encoding nucleotides) will produce IL-17 receptor like polypeptides having functional and chemical characteristics similar to those of naturally occurring IL-17 receptor like polypeptide. In contrast, substantial modifications in the functional and/or chemical characteristics of IL-17 receptor like polypeptides may be accomplished by selecting substitutions in the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide

may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties. It will be appreciated by those of skill in the art that nucleic acid and polypeptide molecules described herein may be chemically synthesized as well as produced by recombinant means.

Naturally occurring residues may be divided into classes based on common side chain properties:

- 1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- 2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- 3) acidic: Asp, Glu;
- 4) basic: His, Lys, Arg;
- 5) residues that influence chain orientation: Gly, Pro; and
- 6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human IL-17 receptor like polypeptide that are homologous with non-human IL-17 receptor like polypeptide orthologs, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine

(+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5);
5 glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., *J. Mol. Biol.*, 157:105-
10 131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are
15 within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of
20 hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent
25 amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine
30 (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8);

isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5);
tryptophan (-3.4). In making changes based upon similar
hydrophilicity values, the substitution of amino acids whose
hydrophilicity values are within ± 2 is preferred, those which
5 are within ± 1 are particularly preferred, and those within
 ± 0.5 are even more particularly preferred. One may also
identify epitopes from primary amino acid sequences on the
basis of hydrophilicity. These regions are also referred to
as "epitopic core regions."

10 Desired amino acid substitutions (whether conservative or
non-conservative) can be determined by those skilled in the
art at the time such substitutions are desired. For example,
amino acid substitutions can be used to identify important
residues of the IL-17 receptor like polypeptide, or to
15 increase or decrease the affinity of the IL-17 receptor like
polypeptides described herein.

Exemplary amino acid substitutions are set forth in Table
I.

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyrlic Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

5 A skilled artisan will be able to determine suitable
variants of the polypeptide as set forth in any of SEQ ID
NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations
thereof, using well known techniques. For identifying
10 suitable areas of the molecule that may be changed without
destroying activity, one skilled in the art may target areas
not believed to be important for activity. For example, when
similar polypeptides with similar activities from the same
species or from other species are known, one skilled in the
15 art may compare the amino acid sequence of an IL-17 receptor
like polypeptide to such similar polypeptides. With such a
comparison, one can identify residues and portions of the
molecules that are conserved among similar polypeptides. It
will be appreciated that changes in areas of an IL-17 receptor
20 like polypeptide that are not conserved relative to such
similar polypeptides would be less likely to adversely affect
the biological activity and/or structure of the IL-17 receptor
like polypeptide. One skilled in the art would also know
that, even in relatively conserved regions, one may substitute
25 chemically similar amino acids for the naturally occurring
residues while retaining activity (conservative amino acid
residue substitutions). Therefore, even areas that may be
important for biological activity or for structure may be
subject to conservative amino acid substitutions without
30 destroying the biological activity or without adversely
affecting the polypeptide structure.

For predicting suitable areas of the molecule that may be
changed without destroying activity, one skilled in the art
may target areas not believed to be important for activity.
30 For example, when similar polypeptides with similar activities
from the same species or from other species are known, one
skilled in the art may compare the amino acid sequence of IL-
17 receptor like polypeptide to such similar polypeptides.

After making such a comparison, one skilled in the art can determine residues and portions of the molecules that are conserved among similar polypeptides. One skilled in the art would know that changes in areas of the IL-17 receptor like molecule that are not conserved would be less likely to adversely affect the biological activity and/or structure of a IL-17 receptor like polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions).

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in an IL-17 receptor like polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of IL-17 receptor like polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of an IL-17 receptor like polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid

substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one
5 discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the
10 amino acids where further substitutions should be avoided either alone or in combination with other mutations.

Numerous scientific publications have been devoted to the prediction of secondary structure, and to the identification of epitopes, from analyses of amino acid sequences. See Chou
15 et al., *Biochemistry*, 13(2):222-245 (1974); Chou et al., *Biochemistry*, 113(2):211-222 (1974); Chou et al., *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou et al., *Ann. Rev. Biochem.*, 47:251-276 and Chou et al., *Biophys. J.*, 26:367-384 (1979). Moreover, computer programs are
20 currently available to assist with predicting antigenic portions and epitopic core regions of proteins. Examples include those programs based upon the . Examples include those programs based upon the Jameson-Wolf analysis (Jameson et al., *Comput. Appl. Biosci.*, 4(1):181-186 (1988) and Wolf et
25 al., *Comput. Appl. Biosci.*, 4(1):187-191 (1988), the program PepPlot® (Brutlag et al., *CABS*, 6:237-245 (1990), and Weinberger et al., *Science*, 228:740-742 (1985), and other new programs for protein tertiary structure prediction (Fetrow et
al., *Biotechnology*, 11:479-483 (1993).

30 Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which

have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain become dramatically in accuracy. more accurate.

Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-9 (1996)), "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Home, supra, and Brenner, supra).

IL-17 receptor like polypeptide analogs of the invention can be determined by comparing the amino acid sequence of IL-17 receptor like polypeptide with related family members. An exemplary IL-17 receptor like polypeptide related family member is human IL-17 receptor polypeptide as set out in SEQ ID NO: 3. This comparison can be accomplished by using a Pileup alignment (Wisconsin GCG Program Package) or an equivalent (overlapping) comparison with multiple family members within conserved and non-conserved regions.

As shown in Figures 2, 4 and 6, the predicted amino acid sequences of human IL-17 receptor like polypeptides (SEQ ID NOS: 2, 5 and 7) are aligned with a known human IL-17 receptor family member (SEQ ID NO: 3) respectively. Other IL-17



receptor like polypeptide analogs can be determined using these or other methods known to those of skill in the art. These overlapping sequences provide guidance for conservative and non-conservative amino acids substitutions resulting in additional IL-17 receptor like analogs. It will be appreciated that these amino acid substitutions can consist of naturally occurring or non-naturally occurring amino acids. For example, potential IL-17 receptor like analogs may have the Met at residue at position 167 of SEQ ID NO: 2, position 225 of SEQ ID NO: 5 or position 50 of SEQ ID NO: 7 substituted with a Leu, Ile, or Phe residue; the Cys residue at position 261 of SEQ ID NO: 2, position 319 of SEQ ID NO: 5 or position 144 of SEQ ID No: 7 substituted with a Ser or Ala residue; and/or the Leu residue at position 299 of SEQ ID NO: 2, position 357 of SEQ ID NO: 5 or position 212 of SEQ ID No: 7 substituted with a norleucine, Gln, Asn, Arg, 1,4, or Diamino-butyric Acid. In addition, potential IL-17 receptor like analogs may have the Trp residue at position 313 of SEQ ID NO: 2, position 371 of SEQ ID NO: 5 or position 196 of SEQ ID NO: 7 substituted with a Tyr or Phe residue; the Gly residue at position 413 of SEQ ID NO: 2, position 471 of SEQ ID NO: 5 or position 296 of SEQ ID NO: 7 substituted with a Pro or Ala residue; and/or the Asp residue at position 433 of SEQ ID NO: 2, position 491 of SEQ ID No: 5 or position 313 of SEQ ID No: 7 substituted with a Glu residue.

Preferred IL-17 receptor like polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites has been altered compared to the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof. In one embodiment, IL-17 receptor like polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites than the amino acid sequence set forth in any of SEQ ID NO:2,

SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution(s) of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred IL-17 receptor like variants include cysteine variants, wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof. Cysteine variants are useful when IL-17 receptor like polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In addition, the polypeptide comprising the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or an IL-17 receptor like polypeptide variant, including a fragment and/or derivative, may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of an IL-17 receptor like fusion polypeptide; a

transmembrane receptor protein or a portion thereof, such as an extracellular domain, or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the polypeptide comprising the amino acid sequence as set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or an IL-17 receptor like polypeptide variant.

Fusions can be made either at the amino terminus or at the carboxy terminus of the polypeptide comprising the amino acid sequence set forth in any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or an IL-17 receptor like polypeptide variant. Fusions may be direct with no linker or adapter molecule or indirect using a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically up to about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

In a further embodiment of the invention, the polypeptide comprising the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, OR SEQ ID NO:7, including combinations thereof, or an IL-17 receptor like polypeptide variant is fused to one or more domains of an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain

known as "Fc", which is involved in effector functions such as complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas an Fab is short-lived. Capon et al., *Nature*, 337:525-31 (1989). When constructed
5 together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein A binding, complement fixation and perhaps even placental transfer. *Id.* Table II summarizes the use of
10 certain Fc fusions known in the art, including materials and methods applicable to the production of fused IL-17 receptor like polypeptide.

2025-01-15 10:20:20

Table II
Fc Fusion with Therapeutic Proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fcγ2a	IL-10	anti-inflammatory; transplant rejection	Zheng et al. (1995), <i>J. Immunol.</i> , <u>154</u> : 5590-5600
IgG1	TNF receptor	septic shock	Fisher et al. (1996), <i>N. Engl. J. Med.</i> , <u>334</u> : 1697-1702; Van Zee et al., (1996), <i>J. Immunol.</i> , <u>156</u> : 2221-2230
IgG, IgA, IgM, or IgE (excluding the first domain)	TNF receptor	inflammation, autoimmune disorders	U.S. Pat. No. 5,808,029, issued September 15, 1998
IgG1	CD4 receptor	AIDS	Capon et al. (1989), <i>Nature</i> <u>337</u> : 525-531
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill et al. (1995), <i>Immunotech.</i> , <u>1</u> : 95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614, published July 3, 1997
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig Cγ1	CTLA-4	autoimmune disorders	Linsley (1991), <i>J. Exp. Med.</i> , <u>174</u> : 561-569

100-10927-0346A
TOP SECRET 22507200

In one example, all or a portion of the human IgG hinge, CH2 and CH3 regions may be fused at either the N-terminus or C-terminus of the IL-17 receptor like polypeptides using methods known to the skilled artisan. In another example, a portion of hinge regions and CH2 and CH3 regions may be fused. The resulting IL-17 receptor like polypeptide-Fc fusion polypeptide may be purified by use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, reduce aggregation, etc.

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al., *SIAM J. Applied Math.*, 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred

computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, *Nucl. Acid. Res.*, 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, *J. Mol. Biol.*, 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (*BLAST Manual*, Altschul *et al.* NCB/NLM/NIH Bethesda, MD 20894; Altschul *et al.*, *supra*). The well known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the

algorithm. A standard comparison matrix (see Dayhoff et al., Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff et al., *Proc. Natl. Acad. Sci USA*, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following:

Algorithm: Needleman et al., *J. Mol. Biol.*, 48:443-453 (1970);

Comparison matrix: BLOSUM 62 from Henikoff et al., *Proc. Natl. Acad. Sci. USA*, 89:10915-10919 (1992);

Gap Penalty: 12

Gap Length Penalty: 4

Threshold of Similarity: 0

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparisons include the following:

Algorithm: Needleman et al., *J. Mol Biol.*, 48:443-453 (1970);

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used,, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

Synthesis

It will be appreciated by those skilled in the art the nucleic acid and polypeptide molecules described herein may be produced by recombinant and other means.

Nucleic Acid Molecules

The nucleic acid molecules encode a polypeptide comprising the amino acid sequence of an IL-17 receptor like polypeptide can readily be obtained in a variety of ways including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening and/or PCR amplification of cDNA.

Recombinant DNA methods used herein are generally those set forth in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and/or Ausubel et al., eds., *Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, NY (1994). The present invention provides for

nucleic acid molecules as described herein and methods for obtaining the molecules.

A gene or cDNA encoding a IL-17 receptor like polypeptide or fragment thereof may be obtained by hybridization screening of a genomic or cDNA library, or by PCR amplification. Where a gene encoding the amino acid sequence of an IL-17 receptor like polypeptide has been identified from one species, all or a portion of that gene may be used as a probe to identify corresponding genes from other species (orthologs) or related genes from the same species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the IL-17 receptor like polypeptide. In addition, part or all of a nucleic acid molecule having the sequence as set forth in any of SEQ ID NO:1, SEQ ID NO:4, OR SEQ ID NO:6, including combinations thereof may be used to screen a genomic library to identify and isolate a gene encoding the amino acid sequence of an IL-17 receptor like polypeptide. Typically, conditions of moderate or high stringency will be employed for screening to minimize the number of false positives obtained from the screen.

Nucleic acid molecules encoding the amino acid sequence of IL-17 receptor like polypeptides may also be identified by expression cloning which employs the detection of positive clones based upon a property of the expressed protein. Typically, nucleic acid libraries are screened by the binding of an antibody or other binding partner (e.g., receptor or ligand) to cloned proteins which are expressed and displayed on a host cell surface. The antibody or binding partner is modified with a detectable label to identify those cells expressing the desired clone.

Recombinant expression techniques conducted in accordance with the descriptions set forth below may be followed to

produce these polynucleotides and to express the encoded polypeptides. For example, by inserting a nucleic acid sequence which encodes the amino acid sequence of an IL-17 receptor like polypeptide into an appropriate vector, one skilled in the art can readily produce large quantities of the desired nucleotide sequence. The sequences can then be used to generate detection probes or amplification primers. Alternatively, a polynucleotide encoding the amino acid sequence of an IL-17 receptor like polypeptide can be inserted into an expression vector. By introducing the expression vector into an appropriate host, the encoded IL-17 receptor like polypeptide may be produced in large amounts.

Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically complementary to two separate regions of cDNA (oligonucleotides) encoding the amino acid sequence of an IL-17 receptor like polypeptide, are then added to the cDNA along with a polymerase such as *Taq* polymerase, and the polymerase amplifies the cDNA region between the two primers.

Another means of preparing a nucleic acid molecule encoding the amino acid sequence of an IL-17 receptor like polypeptide, including a fragment or variant, is chemical synthesis using methods well known to the skilled artisan such as those described by Engels et al., *Angew. Chem. Intl. Ed.*, 28:716-734 (1989). These methods include, *inter alia*, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the amino acid sequence of an IL-17 receptor like polypeptide will be several hundred nucleotides in length.

Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length nucleotide sequence of an IL-17 receptor like polypeptide.

5 Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the IL-17 receptor like polypeptide, depending on whether the polypeptide produced in the host cell is
10 designed to be secreted from that cell. Other methods known to the skilled artisan may be used as well.

In some cases, it may be desirable to prepare nucleic acid molecules encoding IL-17 receptor like polypeptide variants. Nucleic acid molecules encoding variants may be produced using
15 site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired point mutations (see Sambrook et al., supra, and Ausubel et al., supra, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels et al., supra, may
20 also be used to prepare such variants. Other methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have been altered for the optimal expression of an IL-17 receptor like polypeptide in a given host cell.
25 Particular codon alterations will depend upon the IL-17 receptor like polypeptide(s) and host cell(s) selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host
30 cell. Computer algorithms which incorporate codon frequency tables such as "Ecohigh.cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI. Other useful codon frequency tables

include "Celegans_high.cod", "Celegans_low.cod",
"Drosophila_high.cod", "Human_high.cod", "Maize_high.cod", and
"Yeast_high.cod".

In other embodiments, nucleic acid molecules encode IL-17
5 receptor like variants with conservative amino acid
substitutions as described herein, IL-17 receptor like
variants comprising an addition and/or a deletion of one or
more N-linked or O-linked glycosylation sites, IL-17 receptor
like variants having deletions and/or substitutions of one or
10 more cysteine residues, or IL-17 receptor like polypeptide
fragments as described herein. In addition, nucleic acid
molecules may encode any combination of IL-17 receptor like
variants, fragments, and fusion polypeptides described herein.

15 Vectors and Host Cells

A nucleic acid molecule encoding the amino acid sequence
of an IL-17 receptor like polypeptide may be inserted into an
appropriate expression vector using standard ligation
techniques. The vector is typically selected to be functional
20 in the particular host cell employed (i.e., the vector is
compatible with the host cell machinery such that
amplification of the gene and/or expression of the gene can
occur). A nucleic acid molecule encoding the amino acid
sequence of an IL-17 receptor like polypeptide may be
25 amplified/expressed in prokaryotic, yeast, insect (baculovirus
systems), and/or eukaryotic host cells. Selection of the host
cell will depend in part on whether an IL-17 receptor like
polypeptide is to be post-translationally modified (e.g.,
glycosylated and/or phosphorylated). If so, yeast, insect, or
30 mammalian host cells are preferable. For a review of
expression vectors, see *Meth. Enz.*, v.185, D.V. Goeddel, ed.
Academic Press Inc., San Diego, CA (1990).

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, i.e., an oligonucleotide molecule located at the 5' or 3' end of the IL-17 receptor like polypeptide coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or other "tag" such as FLAG, HA (hemagglutinin Influenza virus) or *myc* for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the IL-17 receptor like polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified IL-17 receptor like polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (i.e., from the same species and/or strain as the host cell), heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of flanking sequences from more

than one source) or synthetic, or the flanking sequences may be native sequences which normally function to regulate IL-17 receptor like polypeptide expression. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

The flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein other than the IL-17 receptor like gene flanking sequences will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with suitable oligonucleotide and/or flanking sequence fragments from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of an IL-17 receptor like polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (Product No. 303-3s, New England Biolabs, Beverly, MA) is suitable for most Gram-negative bacteria and various origins (e.g., SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV) or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers

are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

5 Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant
10 cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the selection gene present in
15 the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to the amplification of both the selection gene and the DNA that encodes an IL-17 receptor like
20 polypeptide. As a result, increased quantities of IL-17 receptor like polypeptide are synthesized from the amplified DNA.

A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a
25 Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of an IL-17 receptor like polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a high
30 A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth herein and used in a prokaryotic vector.

A leader, or signal, sequence may be used to direct an IL-17 receptor like polypeptide out of the host cell. Typically, a nucleotide sequence encoding the signal sequence is positioned in the coding region of an IL-17 receptor like nucleic acid molecule, or directly at the 5' end of an IL-17 receptor like polypeptide coding region. Many signal sequences have been identified, and any of those that are functional in the selected host cell may be used in conjunction with an IL-17 receptor like nucleic acid molecule. Therefore, a signal sequence may be homologous (naturally occurring such as amino acids 1 to 14 of SEQ ID NOS: 2 and 5) or heterologous to an IL-17 receptor like gene or cDNA. Additionally, a signal sequence may be chemically synthesized using methods described herein. In most cases, the secretion of an IL-17 receptor like polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the secreted IL-17 receptor like polypeptide. The signal sequence may be a component of the vector, or it may be a part of an IL-17 receptor like nucleic acid molecule that is inserted into the vector.

Included within the scope of this invention is the use of either a nucleotide sequence encoding a native IL-17 receptor like polypeptide signal sequence joined to an IL-17 receptor like polypeptide coding region or a nucleotide sequence encoding a heterologous signal sequence joined to an IL-17 receptor like polypeptide coding region. The heterologous signal sequence selected should be one that is recognized and processed, *i.e.*, cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native IL-17 receptor like polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable

enterotoxin II leaders. For yeast secretion, the native IL-17 receptor like polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal
5 sequence is satisfactory, although other mammalian signal sequences may be suitable.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield.
10 For example, one may alter the peptidase cleavage site of a particular signal peptide, or add presequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to
15 expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the N-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired
20 IL-17 receptor like polypeptide, if the enzyme cuts at such area within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule is increased by the presence of one or more introns in the vector; this is particularly true where a polypeptide is
25 produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the IL-17 receptor like gene, especially where the gene used is a full length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most
30 cDNAs), the intron(s) may be obtained from another source. The position of the intron with respect to flanking sequences and the IL-17 receptor like gene is generally important, as the intron must be transcribed to be effective. Thus, when an

IL-17 receptor like cDNA molecule is being transcribed, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA transcription termination sequence. Preferably, the intron or introns will be located on one side or the other (i.e., 5' or 3') of the cDNA such that it does not interrupt the coding sequence. Any intron from any source, including any viral, prokaryotic and eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell(s) into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

The expression and cloning vectors of the present invention will each typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding a IL-17 receptor like polypeptide. Promoters are untranscribed sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription of the structural gene. Promoters are conventionally grouped into one of two classes, inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, initiate continual gene product production; that is, there is little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding an IL-17 receptor like polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector. The native IL-17

receptor like gene promoter sequence may be used to direct amplification and/or expression of an IL-17 receptor like nucleic acid molecule. A heterologous promoter is preferred, however, if it permits greater transcription and higher yields of the expressed protein as compared to the native promoter, and if it is compatible with the host cell system that has been selected for use.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence(s), using linkers or adapters as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (CMV), a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, e.g., heat-shock promoters and the actin promoter.

Additional promoters which may be of interest in controlling IL-17 receptor like gene transcription include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature*, 290:304-310, 1981); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-

797, 1980); the herpes thymidine kinase promoter (Wagner et al., *Proc. Natl. Acad. Sci. USA*, 78:144-1445, 1981); the regulatory sequences of the metallothionine gene (Brinster et al., *Nature*, 296:39-42, 1982); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff, et al., *Proc. Natl. Acad. Sci. USA*, 75:3727-3731, 1978); or the tac promoter (DeBoer, et al., *Proc. Natl. Acad. Sci. USA*, 80:21-25, 1983). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift et al., *Cell*, 38:639-646, 1984; Ornitz et al., *Cold Spring Harbor Symp. Quant. Biol.*, 50:399-409 (1986); MacDonald, *Hepatology*, 7:425-515, 1987); the insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature*, 315:115-122, 1985); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., *Cell*, 38:647-658 (1984); Adames et al., *Nature*, 318:533-538 (1985); Alexander et al., *Mol. Cell. Biol.*, 7:1436-1444, 1987); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., *Cell*, 45:485-495, 1986); the albumin gene control region which is active in liver (Pinkert et al., *Genes and Devel.*, 1:268-276, 1987); the alphafetoprotein gene control region which is active in liver (Krumlauf et al., *Mol. Cell. Biol.*, 5:1639-1648, 1985; Hammer et al., *Science*, 235:53-58, 1987); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., *Genes and Devel.*, 1:161-171, 1987); the beta-globin gene control region which is active in myeloid cells (Mogram et al., *Nature*, 315:338-340, 1985; Kollias et al., *Cell*, 46:89-94, 1986); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., *Cell*, 48:703-712, 1987);

the myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature*, 314:283-286, 1985); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., *Science*, 234:1372-1378, 1986).

An enhancer sequence may be inserted into the vector to increase the transcription of a DNA encoding an IL-17 receptor like polypeptide of the present invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent. They have been found 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to an IL-17 receptor like nucleic acid molecule, it is typically located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the desired flanking sequences are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3,

and pcDNA3.1 (Invitrogen Company, Carlsbad, CA), pBSII (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-
5 alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids, plasmids or modified viruses, but it will be appreciated that the vector system must be compatible with the
10 selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript[®] plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO[™] TA Cloning[®] Kit,
15 PCR2.1[®] plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast, or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). The recombinant molecules can be introduced into host cells via transformation, transfection, infection, or
20 other known techniques.

After the vector has been constructed and a nucleic acid molecule encoding an IL-17 receptor like polypeptide has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for
25 amplification and/or polypeptide expression. The transformation of an expression vector for an IL-17 receptor like polypeptide into a selected host cell may be accomplished by well known methods including methods such as transfection, infection, calcium chloride, electroporation, microinjection,
30 lipofection or the DEAE-dextran method or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other

suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook et al., *supra*.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell or a vertebrate cell). The host cell, when cultured under appropriate conditions, synthesizes an IL-17 receptor like polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. Examples include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR-cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA*, 97:4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate cells

may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines
5 derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the ATCC. Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present
10 invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, (ATCC No. 33694) DH5 α , DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and
15 the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia*
20 *pastoris*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts et al., *Biotechniques*, 14:810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, 4:564-572 (1993); and Lucklow et al. (*J. Virol.*, 67:4566-4579 (1993)). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).
25

One may also use transgenic animals to express glycosylated IL-17 receptor like polypeptides. For example,
30 one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide

in the animal milk. One may also use plants to produce IL-17
receptor like polypeptides, however, in general, the
glycosylation occurring in plants is different from that
produced in mammalian cells, and may result in a glycosylated
5 product which is not suitable for human therapeutic use.

Polypeptide Production

Host cells comprising an IL-17 receptor like polypeptide
expression vector may be cultured using standard media well
10 known to the skilled artisan. The media will usually contain
all nutrients necessary for the growth and survival of the
cells. Suitable media for culturing *E. coli* cells include,
for example, Luria Broth (LB) and/or Terrific Broth (TB).
Suitable media for culturing eukaryotic cells include Roswell
15 Park Memorial Institute medium 1640 (RPMI 1640), Minimal
Essential Medium (MEM) and/or Dulbecco's Modified Eagle Medium
(DMEM), all of which may be supplemented with serum and/or
growth factors as indicated by the particular cell line being
cultured. A suitable medium for insect cultures is Grace's
20 medium supplemented with yeastolate, lactalbumin hydrolysate
and/or fetal calf serum, as necessary.

Typically, an antibiotic or other compound useful for
selective growth of transformed cells is added as a supplement
to the media. The compound to be used will be dictated by the
25 selectable marker element present on the plasmid with which
the host cell was transformed. For example, where the
selectable marker element is kanamycin resistance, the
compound added to the culture medium will be kanamycin. Other
compounds for selective growth include ampicillin,
30 tetracycline, and neomycin.

The amount of an IL-17 receptor like polypeptide produced
by a host cell can be evaluated using standard methods known

in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, high performance liquid chromatography (HPLC) separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If an IL-17 receptor like polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. If however, the IL-17 receptor like polypeptide is not secreted from the host cells, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells). The host cells are typically disrupted mechanically or with a detergent to release the intracellular contents into a buffered solution. IL-17 receptor like polypeptide can then be isolated from the solution.

For an IL-17 receptor like polypeptide situated in the host cell cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells), intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

If an IL-17 receptor like polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with a chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in

the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The IL-17 receptor like polypeptide in its now soluble form
5 can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate the IL-17 receptor like polypeptide, isolation may be accomplished using standard methods such as those described herein and in Marston et al., *Meth. Enz.*, 182:264-275 (1990).

10 In some cases, an IL-17 receptor like polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological activity. Such methods include exposing
15 the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same
20 as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of
25 the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol (DTT)/dithiane DTT, and 2-2mercaptoethanol (β ME)/dithio- β (ME). A cosolvent may be used to increase the efficiency of the
30 refolding, and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

If inclusion bodies are not formed to a significant degree upon expression of an IL-17 receptor like polypeptide, then the polypeptide will be found primarily in the supernatant after centrifugation of the cell homogenate. The polypeptide may be further isolated from the supernatant using methods such as those described herein.

The purification of an IL-17 receptor like polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (IL-17 receptor like polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen, Carlsbad, CA) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of IL-17 receptor like polypeptide/polyHis. See for example, Ausubel et al., eds., *Current Protocols in Molecular Biology*, Section 10.11.8, John Wiley & Sons, New York (1993).

Additionally, the IL-17 receptor like polypeptide may be purified through the use of a monoclonal antibody which is capable of specifically recognizing and binding to the IL-17 receptor like polypeptide.

Suitable procedures for purification thus include, without limitation, affinity chromatography, immunoaffinity chromatography, ion exchange chromatography, molecular sieve chromatography, High Performance Liquid Chromatography (HPLC), electrophoresis (including native gel electrophoresis)

followed by gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific, San Francisco, CA). In some cases, two or more purification techniques may be combined to achieve increased purity.

5 IL-17 receptor like polypeptides, including fragments, variants and/or derivatives thereof may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art, such as those set forth by Merrifield et al., *J. Am. Chem. Soc.*, 85:2149
10 (1963), Houghten et al., *Proc Natl Acad. Sci. USA*, 82:5132 (1985), and Stewart and Young, *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL (1984). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized IL-17 receptor like
15 polypeptides may be oxidized using methods set forth in these references to form disulfide bridges. Chemically synthesized IL-17 receptor like polypeptides are expected to have comparable biological activity to the corresponding IL-17 receptor like polypeptides produced recombinantly or purified
20 from natural sources, and thus may be used interchangeably with a recombinant or natural IL-17 receptor like polypeptide.

Another means of obtaining an IL-17 receptor like polypeptide is via purification from biological samples such as source tissues and/or fluids in which the IL-17 receptor
25 like polypeptide is naturally found. Such purification can be conducted using methods for protein purification as described herein. The presence of the IL-17 receptor like polypeptide during purification may be monitored using, for example, an antibody prepared against recombinantly produced IL-17
30 receptor like polypeptide or peptide fragments thereof.

A number of additional methods for producing nucleic acids and polypeptides are known in the art, and the methods

can be used to produce polypeptides having specificity for IL-17 receptor like. See for example, Roberts et al., *Proc. Natl. Acad. Sci. U.S.A.*, 94:12297-12303 (1997), which describes the production of fusion proteins between an mRNA and its encoded peptide. See also Roberts, R., *Curr. Opin. Chem. Biol.*, 3:268-273 (1999). Additionally, U.S. patent No. 5,824,469 describes methods of obtaining oligonucleotides capable of carrying out a specific biological function. The procedure involves generating a heterogeneous pool of oligonucleotides, each having a 5' randomized sequence, a central preselected sequence, and a 3' randomized sequence. The resulting heterogeneous pool is introduced into a population of cells that do not exhibit the desired biological function. Subpopulations of the cells are then screened for those which exhibit a predetermined biological function. From that subpopulation, oligonucleotides capable of carrying out the desired biological function are isolated.

U.S. Patent Nos. 5,763,192, 5,814,476, 5,723,323, and 5,817,483 describe processes for producing peptides or polypeptides. This is done by producing stochastic genes or fragments thereof, and then introducing these genes into host cells which produce one or more proteins encoded by the stochastic genes. The host cells are then screened to identify those clones producing peptides or polypeptides having the desired activity.

Another method for producing peptides or polypeptides is described in PCT/US98/20094 (WO99/15650) filed by Athersys, Inc. Known as "Random Activation of Gene Expression for Gene Discovery" (RAGE-GD), the process involves the activation of endogenous gene expression or over-expression of a gene by in situ recombination methods. For example, expression of an endogenous gene is activated or increased by integrating a regulatory sequence into the target cell which is capable of

activating expression of the gene by non-homologous or illegitimate recombination. The target DNA is first subjected to radiation, and a genetic promoter inserted. The promoter eventually locates a break at the front of a gene, initiating transcription of the gene. This results in expression of the desired peptide or polypeptide.

It will be appreciated that these methods can also be used to create comprehensive IL-17 receptor like protein expression libraries, which can subsequently be used for high throughput phenotypic screening in a variety of assays, such as biochemical assays, cellular assays, and whole organism assays (e.g., plant, mouse, etc.).

Chemical Derivatives

Chemically modified derivatives of the IL-17 receptor like polypeptides may be prepared by one skilled in the art, given the disclosures set forth hereinbelow. IL-17 receptor like polypeptide derivatives are modified in a manner that is different, either in the type or location of the molecules naturally attached to the polypeptide. Derivatives may include molecules formed by the deletion of one or more naturally-attached chemical groups. The polypeptide comprising the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or an IL-17 receptor like polypeptide variant may be modified by the covalent attachment of one or more polymers. For example, the polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Included within the scope of suitable polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

The polymers each may be of any molecular weight and may be branched or unbranched. The polymers each typically have an average molecular weight of between about 2kDa to about 100kDa (the term "about" indicating that in preparations of a water soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer preferably is between about 5kDa and about 50kDa, more preferably between about 12kDa and about 40kDa and most preferably between about 20kDa and about 35kDa.

Suitable water soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C₁-C₁₀) alkoxy- or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran, of, for example about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules which may be used to prepare covalently attached multimers of the polypeptide comprising the amino acid sequence of any of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:7, including combinations thereof, or an IL-17 receptor like polypeptide variant.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemical derivatives of polypeptides will generally comprise the steps of (a) reacting the polypeptide with the activated polymer

5 molecule (such as a reactive ester or aldehyde derivative of
the polymer molecule) under conditions whereby the polypeptide
comprising the amino acid sequence of any of SEQ ID NO:2, SEQ
ID NO:5, or SEQ ID NO:7, including combinations thereof, or an
10 IL-17 receptor like polypeptide variant becomes attached to
one or more polymer molecules, and (b) obtaining the reaction
product(s). The optimal reaction conditions will be
determined based on known parameters and the desired result.
For example, the larger the ratio of polymer
15 molecules:protein, the greater the percentage of attached
polymer molecule. In one embodiment, the IL-17 receptor like
polypeptide derivative may have a single polymer molecule
moiety at the amino terminus. See, for example, U.S. Patent
No. 5,234,784.

20 The pegylation of the polypeptide specifically may be
carried out by any of the pegylation reactions known in the
art, as described for example in the following references:
Francis et al., *Focus on Growth Factors*, 3:4-10 (1992); EP
0154316; EP 0401384 and U.S. Patent No. 4,179,337. For
25 example, pegylation may be carried out via an acylation
reaction or an alkylation reaction with a reactive
polyethylene glycol molecule (or an analogous reactive water-
soluble polymer) as described herein. For the acylation
reactions, the polymer(s) selected should have a single
30 reactive ester group. For reductive alkylation, the
polymer(s) selected should have a single reactive aldehyde
group. A reactive aldehyde is, for example, polyethylene
glycol propionaldehyde, which is water stable, or mono C₁-C₁₀
alkoxy or aryloxy derivatives thereof (see U.S. Patent No.
5,252,714).

In another embodiment, IL-17 receptor like polypeptides
may be chemically coupled to biotin, and the biotin/IL-17

receptor like polypeptide molecules which are conjugated are then allowed to bind to avidin, resulting in tetravalent avidin/biotin/IL-17 receptor like polypeptide molecules. IL-17 receptor like polypeptides may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates with a valency of 10.

Generally, conditions which may be alleviated or modulated by the administration of the present IL-17 receptor like polypeptide derivatives include those described herein for IL-17 receptor like polypeptides. However, the IL-17 receptor like polypeptide derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to the non-derivatized molecules.

Genetically Engineered Non-Human Animals

Additionally included within the scope of the present invention are non-human animals such as mice, rats, or other rodents, rabbits, goats, or sheep, or other farm animals, in which the gene (or genes) encoding the native IL-17 receptor like polypeptide has (have) been disrupted ("knocked out") such that the level of expression of this gene or genes is (are) significantly decreased or completely abolished. Such animals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032.

The present invention further includes non-human animals such as mice, rats, or other rodents, rabbits, goats, sheep, or other farm animals, in which either the native form of the IL-17 receptor like gene(s) for that animal or a heterologous IL-17 receptor like gene(s) is (are) over-expressed by the

animal, thereby creating a "transgenic" animal. Such transgenic animals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT application No. WO94/28122.

5 The present invention further includes non-human animals in which the promoter for one or more of the IL-17 receptor like polypeptides of the present invention is either activated or inactivated (e.g., by using homologous recombination methods) to alter the level of expression of one or more of
10 the native IL-17 receptor like polypeptides.

These non-human animals may be used for drug candidate screening. In such screening, the impact of a drug candidate on the animal may be measured. For example, drug candidates may decrease or increase the expression of the IL-17 receptor
15 like gene. In certain embodiments, the amount of IL-17 receptor like polypeptide, that is produced may be measured after the exposure of the animal to the drug candidate. Additionally, in certain embodiments, one may detect the actual impact of the drug candidate on the animal. For
20 example, the overexpression of a particular gene may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease expression of the gene or its ability to prevent or inhibit a pathological condition. In other
25 examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product or its ability to
30 prevent or inhibit a pathological condition.

Microarray

It will be appreciated that DNA microarray technology can be utilized in accordance with the present invention. DNA microarrays are miniature, high density arrays of nucleic acids positioned on a solid support, such as glass. Each cell or element within the array has numerous copies of a single species of DNA which acts as a target for hybridization for its cognate mRNA. In expression profiling using DNA microarray technology, mRNA is first extracted from a cell or tissue sample and then converted enzymatically to fluorescently labeled cDNA. This material is hybridized to the microarray and unbound cDNA is removed by washing. The expression of discrete genes represented on the array is then visualized by quantitating the amount of labeled cDNA which is specifically bound to each target DNA. In this way, the expression of thousands of genes can be quantitated in a high throughput, parallel manner from a single sample of biological material.

This high throughput expression profiling has a broad range of applications with respect to the IL-17 receptor like molecules of the invention, including, but not limited to: the identification and validation of IL-17 receptor like disease-related genes as targets for therapeutics; molecular toxicology of IL-17 receptor like molecules and inhibitors thereof; stratification of populations and generation of surrogate markers for clinical trials; and enhancing IL-17 receptor like-related small molecule drug discovery by aiding in the identification of selective compounds in high throughput screens (HTS).

Selective Binding Agents

As used herein, the term "selective binding agent" refers to a molecule which has specificity for one or more IL-17 receptor like polypeptides. Suitable selective binding agents include, but are not limited to, antibodies and derivatives thereof, polypeptides, and small molecules. Suitable selective binding agents may be prepared using methods known in the art. An exemplary IL-17 receptor like polypeptide selective binding agent of the present invention is capable of binding a certain portion of the IL-17 receptor like polypeptide thereby inhibiting the binding of a ligand such as IL17E of SEQ ID NO: 23 to the IL-17 receptor like polypeptide receptor(s).

Selective binding agents such as antibodies and antibody fragments that bind IL-17 receptor like polypeptides are within the scope of the present invention. The antibodies may be polyclonal including monospecific polyclonal, monoclonal (MAbs), recombinant, chimeric, humanized such as CDR-grafted, human, single chain, and/or bispecific, as well as fragments, variants or derivatives thereof. Antibody fragments include those portions of the antibody which bind to an epitope on the IL-17 receptor like polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies directed toward an IL-17 receptor like polypeptide generally are produced in animals (e.g., rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of IL-17 receptor like polypeptide and an adjuvant. It may be useful to conjugate an IL-17

receptor like polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-IL-17 receptor like polypeptide antibody titer.

Monoclonal antibodies directed toward an IL-17 receptor like polypeptide are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler et al., *Nature*, 256:495-497 (1975) and the human B-cell hybridoma method, Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987). Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with IL-17 receptor like polypeptides.

Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity. See, U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1985).

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. See U.S. Patent Nos. 5,585,089, and 5,693,762. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed, for example, using methods described in the art. (See U.S. Patent Nos. 5,585,089 and 5,693,762). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed, for example, using methods known in the art (Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science* 239:1534-1536 (1988)), by substituting at least a portion of a rodent complementarity-determining regions (CDRs) for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies which bind IL-17 receptor like polypeptides. Using transgenic animals (e.g., mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production such antibodies are produced by immunization with an IL-17 receptor like antigen (i.e., having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, for example, Jakobovits et al., *Proc. Natl. Acad. Sci.*, 90:2551-2555 (1993); Jakobovits et al., *Nature* 362:255-258 (1993); Bruggermann et al., *Year in Immuno.*, 7:33 (1993). In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, that is those having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired

immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human variable regions including human (rather than e.g., murine) amino acid sequences, including variable regions, including human which are immunospecific for these antigens. See PCT application nos. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT application nos. PCT/US91/245, PCT/GB89/01207, and in EP 546073B1 and EP 546073A1. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In an alternative embodiment, human antibodies can be produced from phage-display libraries (Hoogenboom et al., *J. Mol. Biol.* 227:381 (1991); Marks et al., *J. Mol. Biol.* 222:581 (1991). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Application no. PCT/US98/17364, which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk- receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (e.g., human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-IL-17 receptor like antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Sola, Monoclonal Antibodies: A
5 Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987)) for the detection and quantitation of IL-17 receptor like polypeptides. The antibodies will bind IL-17 receptor like polypeptides with an affinity which is appropriate for the assay method being employed.

10 For diagnostic applications, in certain embodiments, anti-IL-17 receptor like antibodies may be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be
15 a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β -galactosidase, or horseradish peroxidase (Bayer et al., *Meth. Enz.*, 184:138-163 (1990)).

20 Competitive binding assays rely on the ability of a labeled standard (e.g., an IL-17 receptor like polypeptide, or an immunologically reactive portion thereof) to compete with the test sample analyte (an IL-17 receptor like polypeptide) for binding with a limited amount of anti IL-17 receptor like
25 antibody. The amount of an IL-17 receptor like polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or after the
30 competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex. See, e.g., U.S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assays). For example, one type of sandwich assay is an enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The selective binding agents, including anti-IL-17 receptor like antibodies, also are useful for *in vivo* imaging. An antibody labeled with a detectable moiety may be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. The antibody may be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

The invention also relates to a kit comprising IL-17 receptor like selective binding agents (such as antibodies) and other reagents useful for detecting IL-17 receptor like polypeptide levels in biological samples. Such reagents may include a secondary activity, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

Selective binding agents of the invention, including antibodies, may be used as therapeutics. These therapeutic

agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of an IL-17 receptor like polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to an IL-17 receptor like polypeptide and which are capable of inhibiting or eliminating the functional activity of an IL-17 receptor like polypeptide *in vivo* or *in vitro*. In preferred embodiments, the selective binding agent, e.g., an antagonist antibody, will inhibit the functional activity of an IL-17 receptor like polypeptide by at least about 50%, and preferably by at least about 80%. In another embodiment, the selective binding agent may be an anti-IL-17 receptor like polypeptide antibody that is capable of interacting with an IL-17 receptor like binding partner (a ligand or receptor) thereby inhibiting or eliminating IL-17 receptor like activity *in vitro* or *in vivo*. Selective binding agents, including agonist and antagonist anti-IL-17 receptor like antibodies, are identified by screening assays which are well known in the art.

The invention also relates to a kit comprising IL-17 receptor like selective binding agents (such as antibodies) and other reagents useful for detecting IL-17 receptor like polypeptide levels in biological samples. Such reagents may include, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

IL-17 receptor like polypeptides can be used to clone IL-17 receptor like ligand(s) using an "expression cloning" strategy. Radiolabeled (¹²⁵-Iodine) IL-17 receptor like polypeptide or "affinity/activity-tagged" IL-17 receptor like polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type

or cell line or tissue that expresses IL-17 receptor like
ligand(s). RNA isolated from such cells or tissues can then
be converted to cDNA, cloned into a mammalian expression
vector, and transfected into mammalian cells (for example,
5 COS, or 293) to create an expression library. Radiolabeled or
tagged IL-17 receptor like polypeptide can then be used as an
affinity reagent to identify and isolate the subset of cells
in this library expressing IL-17 receptor like ligand(s). DNA
is then isolated from these cells and transfected into
10 mammalian cells to create a secondary expression library in
which the fraction of cells expressing IL-17 receptor like
ligand(s) would be many-fold higher than in the original
library. This enrichment process can be repeated iteratively
until a single recombinant clone containing an IL-17 receptor
15 like ligand is isolated. Isolation of IL-17 receptor like
ligand(s) is useful for identifying or developing novel
agonists and antagonists of the IL-17 receptor like signaling
pathway. Such agonists and antagonists include IL-17 receptor
like ligand(s), anti-IL-17 receptor like ligand antibodies,
20 small molecules, or antisense oligonucleotides.

Assaying for Other Modulators of IL-17 Receptor Like Polypeptide Activity

In some situations, it may be desirable to identify
25 molecules that are modulators, i.e., agonists or antagonists,
of the activity of IL-17 receptor like polypeptide. Natural
or synthetic molecules that modulate IL-17 receptor like
polypeptide may be identified using one or more screening
assays, such as those described herein. Such molecules may be
30 administered either in an ex vivo manner, or in an in vivo
manner by injection, or by oral delivery, implantation device,
or the like.

10340927 031504
10340927 031504

"Test molecule(s)" refers to the molecule(s) that is/are under evaluation for the ability to modulate (i.e., increase or decrease) the activity of an IL-17 receptor like polypeptide. Most commonly, a test molecule will interact directly with an IL-17 receptor like polypeptide. However, it is also contemplated that a test molecule may also modulate IL-17 receptor like polypeptide activity indirectly, such as by affecting IL-17 receptor like gene expression, or by binding to an IL-17 receptor like binding partner (e.g., receptor or ligand). In one embodiment, a test molecule will bind to an IL-17 receptor like polypeptide with an affinity constant of at least about 10^{-6} M, preferably about 10^{-8} M, more preferably about 10^{-9} M, and even more preferably about 10^{-10} M.

Methods for identifying compounds which interact with IL-17 receptor like polypeptides are encompassed by the present invention. In certain embodiments, an IL-17 receptor like polypeptide is incubated with a test molecule under conditions which permit the interaction of the test molecule with an IL-17 receptor like polypeptide, and the extent of the interaction can be measured. The test molecule(s) can be screened in a substantially purified form or in a crude mixture. The test molecules can be nucleic acid molecules, proteins, peptides, carbohydrates, lipids, organic and inorganic compounds.

In certain embodiments, an IL-17 receptor like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with IL-17 receptor like polypeptide, or ligand thereof, to regulate its activity. Molecules which regulate IL-17 receptor like polypeptide expression include nucleic acids which are complementary to nucleic acids encoding an IL-

17 receptor like polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of IL-17 receptor like polypeptide, and which act as anti-sense regulators of expression.

5 Once a set of test molecules has been identified as interacting with an IL-17 receptor like polypeptide, the molecules may be further evaluated for their ability to increase or decrease IL-17 receptor like polypeptide activity. The measurement of the interaction of test molecules with IL-17 receptor like polypeptides may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. In general, test molecules are incubated with an IL-17 receptor like polypeptide for a specified period of time, and IL-17 receptor like polypeptide activity is determined by one or more assays for measuring biological activity.

10 The interaction of test molecules with IL-17 receptor like polypeptides may also be assayed directly using polyclonal or monoclonal antibodies in an immunoassay. Alternatively, modified forms of IL-17 receptor like polypeptides containing epitope tags as described herein may be used in immunoassays.

15 In certain embodiments, a IL-17 receptor like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with IL-17 receptor like polypeptide to regulate its activity. Potential protein antagonists of IL-17 receptor like polypeptide include antibodies which interact with active regions of the polypeptide and inhibit or eliminate at least one activity of IL-17 receptor like molecules. Molecules which regulate IL-17 receptor like polypeptide expression include nucleic acids which are complementary to nucleic acids

encoding a IL-17 receptor like polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of IL-17 receptor like polypeptide, and which act as anti-sense regulators of expression.

5 In the event that IL-17 receptor like polypeptides display biological activity through an interaction with a binding partner (e.g., a receptor or a ligand), a variety of *in vitro* assays may be used to measure the binding of an IL-17 receptor like polypeptide to the corresponding binding partner
10 (such as a selective binding agent, receptor, or ligand). These assays may be used to screen test molecules for their ability to increase or decrease the rate and/or the extent of binding of an IL-17 receptor like polypeptide to its binding partner. In one assay, an IL-17 receptor like polypeptide is
15 immobilized in the wells of a microtiter plate. Radiolabeled IL-17 receptor like binding partner (for example, iodinated IL-17 receptor like binding partner) and the test molecule(s) can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can
20 be washed and counted, using a scintillation counter, for radioactivity to determine the extent to which the binding partner bound to IL-17 receptor like polypeptide. Typically, the molecules will be tested over a range of concentrations, and a series of control wells lacking one or more elements of
25 the test assays can be used for accuracy in the evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins, i.e., immobilizing IL-17 receptor like binding partner to the microtiter plate wells, incubating with the test molecule and radiolabeled IL-17
30 receptor like polypeptide, and determining the extent of IL-17 receptor like polypeptide binding. See, for example, chapter 18, *Current Protocols in Molecular Biology*, Ausubel et al., eds., John Wiley & Sons, New York, NY (1995).

As an alternative to radiolabelling, an IL-17 receptor like polypeptide or its binding partner may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), that can be detected colorometrically, or by fluorescent tagging of streptavidin. An antibody directed to an IL-17 receptor like polypeptide or to an IL-17 receptor like binding partner and conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP.

An IL-17 receptor like polypeptide or an IL-17 receptor like binding partner can also be immobilized by attachment to agarose beads, acrylic beads or other types of such inert solid phase substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound. After incubation, the beads can be precipitated by centrifugation, and the amount of binding between an IL-17 receptor like polypeptide and its binding partner can be assessed using the methods described herein. Alternatively, the substrate-protein complex can be immobilized in a column, and the test molecule and complementary protein are passed through the column. The formation of a complex between an IL-17 receptor like polypeptide and its binding partner can then be assessed using any of the techniques set forth herein, i.e., radiolabelling, antibody binding, or the like.

Another *in vitro* assay that is useful for identifying a test molecule which increases or decreases the formation of a complex between an IL-17 receptor like binding protein and an IL-17 receptor like binding partner is a surface plasmon resonance detector system such as the BIAcore assay system

(Pharmacia, Piscataway, NJ). The BIAcore system may be carried out using the manufacturer's protocol. This assay essentially involves the covalent binding of either IL-17 receptor like polypeptide or an IL-17 receptor like binding partner to a dextran-coated sensor chip which is located in a detector. The test compound and the other complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test compounds together for their ability to increase or decrease the formation of a complex between an IL-17 receptor like polypeptide and an IL-17 receptor like binding partner. In these cases, the assays set forth herein can be readily modified by adding such additional test compound(s) either simultaneous with, or subsequent to, the first test compound. The remainder of the steps in the assay are as set forth herein.

In vitro assays such as those described herein may be used advantageously to screen large numbers of compounds for effects on complex formation by IL-17 receptor like polypeptide and IL-17 receptor like binding partner. The assays may be automated to screen compounds generated in phage display, synthetic peptide, and chemical synthesis libraries.

Compounds which increase or decrease the formation of a complex between an IL-17 receptor like polypeptide and an IL-17 receptor like binding partner may also be screened in cell culture using cells and cell lines expressing either IL-17

receptor like polypeptide or IL-17 receptor like binding partner. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of an IL-17 receptor like polypeptide to cells expressing IL-17 receptor like binding partner at the surface is evaluated in the presence or absence of test molecules, and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to an IL-17 receptor like binding partner. Cell culture assays can be used advantageously to further evaluate compounds that score positive in protein binding assays described herein.

Cell cultures can also be used to screen the impact of a drug candidate. For example, drug candidates may decrease or increase the expression of the IL-17 receptor like gene. In certain embodiments, the amount of IL-17 receptor like polypeptide that is produced may be measured after exposure of the cell culture to the drug candidate. In certain embodiments, one may detect the actual impact of the drug candidate on the cell culture. For example, the overexpression of a particular gene may have a particular impact on the cell culture. In such cases, one may test a drug candidate's ability to increase or decrease the expression of the gene or its ability to prevent or inhibit a particular impact on the cell culture. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product in a cell culture.

A yeast two-hybrid system (Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-9583, 1991) can be used to identify

novel polypeptides that bind to, or interact with, IL-17
receptor like polypeptides. As an example, a yeast-two hybrid
bait construct can be generated in a vector (such as the pAS2-
1 from Clontech) which encodes a yeast GAL4-DNA binding domain
5 fused to the Cdk11 polynucleotide. This bait construct may be
used to screen human cDNA libraries wherein the cDNA library
sequences are fused to GAL4 activation domains. Positive
interactions will result in the activation of a reporter gene
such as β -Gal. Positive clones emerging from the screening
10 may be characterized further to identify interacting proteins.

Internalizing Proteins

The *tat* protein sequence (from HIV) can be used to
internalize proteins into a cell. See e.g., Falwell et al.,
Proc. Natl. Acad. Sci., 91:664-668 (1994). For example, an 11
15 amino acid sequence (YGRKKRRQRRR SEQ ID NO: 18) of the HIV *tat*
protein (termed the "protein transduction domain", or TAT PDT)
has been described as mediating delivery across the
cytoplasmic membrane and the nuclear membrane of a cell. See
Schwarze et al., *Science*, 285:1569-1572 (1999); and Nagahara
20 et al., *Nature Medicine*, 4:1449-1452 (1998). In these
procedures, FITC-constructs (FITC-GGGGYGRKKRRQRRR SEQ ID NO:
19) are prepared which bind to cells as observed by
fluorescence-activated cell sorting (FACS) analysis, and these
constructs penetrate tissues after i.p. administration. Next,
25 *tat*-bgal fusion proteins are constructed. Cells treated with
this construct demonstrated β -gal activity. Following
injection, a number of tissues, including liver, kidney, lung,
heart, and brain tissue have been found to demonstrate
expression using these procedures. It is believed that these
30 constructions underwent some degree of unfolding in order to
enter the cell; as such, refolding may be required after
entering the cell.

It will thus be appreciated that the tat protein sequence may be used to internalize a desired protein or polypeptide into a cell. For example, using the tat protein sequence, an IL-17 receptor like antagonist (such as an anti-IL-17 receptor like selective binding agent, small molecule, soluble receptor, or antisense oligonucleotide) can be administered intracellularly to inhibit the activity of an IL-17 receptor like molecule. As used herein, the term "IL-17 receptor like molecule" refers to both IL-17 receptor like nucleic acid molecules and IL-17 receptor like polypeptides as defined herein. Where desired, the IL-17 receptor like protein itself may also be internally administered to a cell using these procedures. See also, Strauss, E., "Introducing Proteins Into the Body's Cells", *Science*, 285:1466-1467 (1999).

Cell Source Identification Using IL-17 Receptor Like Polypeptides

In accordance with certain embodiments of the invention, it may be useful to be able to determine the source of a certain cell type associated with an IL-17 receptor like polypeptide. For example, it may be useful to determine the origin of a disease or pathological condition as an aid in selecting an appropriate therapy.

Therapeutic Uses

A non-exclusive list of acute and chronic diseases which can be treated, diagnosed, ameliorated, or prevented with the IL-17 receptor like nucleic acids, polypeptides, and agonists and antagonists of the invention include:

- 5 • The diagnosis and/or treatment of diseases involving immune system dysfunction. Examples of such diseases include, but are not limited to, rheumatoid arthritis, psoriatic arthritis, inflammatory arthritis, osteoarthritis, inflammatory joint disease, autoimmune disease including autoimmune vasculitis, multiple sclerosis, lupus, diabetes (e.g., insulin diabetes), inflammatory bowel disease, transplant rejection, graft vs. host disease, and inflammatory conditions resulting from strain, sprain, cartilage damage, trauma, orthopedic surgery, infection or other disease processes. Other diseases influenced by the dysfunction of the immune system are encompassed within the scope of the invention, including but not limited to, allergies. The IL-17 receptor like nucleic acids, polypeptides, and agonists and antagonists of the invention can also be used to inhibit T cell proliferation, to inhibit T cell activation, and/or to inhibit B cell proliferation and/or immunoglobulin secretion.
- 10
- 15
- 20 • The diagnosis and/or treatment of diseases involving infection. Examples of such diseases include, but are not limited to, leprosy, viral infections such as hepatitis or HIV, bacterial infection such as *clostridium* associated illnesses, including *clostridium*-associated diarrhea, pulmonary tuberculosis, acute febrile illness from bacteria such as or virus, fever, acute phase response of the liver, septicemia, septic shock. Other diseases involving infection are encompassed within the scope of the invention.
- 25
- 30 • The diagnosis and/or treatment of diseases involving weight disorders. Examples of such diseases include, but are not limited to obesity, anorexia, cachexia, including

AIDS-induced cachexia, myopathies (e.g., muscle protein metabolism, such as in sepsis), and hypoglycemia. Other diseases involving weight disorders are encompassed within the scope of the invention.

- 5 • The diagnosis and/or treatment of diseases involving neuronal dysfunction. Examples of such diseases include, but are not limited to Alzheimer's, Parkinson's disease, neurotoxicity (e.g., as induced by HIV), ALS, brain injury, stress, depression, nociception and other pain
10 (including cancer-related pain), hyperalgesia, epilepsy, learning impairment and memory disorders, sleep disturbance, and peripheral and central neuropathies. Other neurological disorders are encompassed within the scope of the invention.

- 15 • The diagnosis and/or treatment of diseases involving the lung. Examples of such diseases include, but are not limited to, acute or chronic lung injury including interstitial lung disease, acute respiratory disease syndrome, pulmonary hypertension, emphysema, cystic
20 fibrosis, pulmonary fibrosis, and asthma. Other diseases of the lung are encompassed within the scope of the invention.

- 25 • The diagnosis and/or treatment of diseases involving the skin. Examples of such diseases include, but are not limited to, psoriasis, eczema, and wound healing. Other diseases of the skin are encompassed within the scope of the Invention.

- 30 • The diagnosis and/or treatment of diseases involving the kidney. Examples of such diseases include, but are not limited to, acute and chronic glomerulonephritis. Other diseases of the kidney are encompassed within the scope

of the invention.

- The diagnosis and/or treatment of diseases involving the bone. Examples of such diseases include, but are not limited to, osteoporosis, osteopetrosis, osteogenesis imperfecta, Paget's disease, periodontal disease, temporal mandibular joint disease, and hypercalcemia. Other diseases of the bone are encompassed within the scope of the invention.
- The diagnosis and/or treatment of diseases involving the vascular system. Examples of such diseases include, but are not limited to hemorrhage or stroke, hemorrhagic shock, ischemia, including cardiac ischemia and cerebral ischemia (e.g., brain injury as a result of trauma, epilepsy, hemorrhage or stroke, each of which may lead to neurodegeneration), atherosclerosis, congestive heart failure; restenosis, reperfusion injury, and angiogenesis. Other diseases of the vascular system are encompassed within the scope of the invention.
- The diagnosis and/or treatment of tumor cells. Examples of such diseases include, but are not limited to, lymphomas, bone sarcoma, chronic and acute myelogenous leukemia (AML and CML), myelomonocytic leukemias and other leukemias, multiple myeloma, lung, breast cancer, tumor metastasis, and side effects from radiation therapy. Other diseases involving tumor cells are encompassed within the scope of the invention.
- The diagnosis and/or treatment of reproductive disorders. Examples of such diseases include, but are not limited to, infertility, miscarriage, pre-term labor and delivery, and endometriosis. Other diseases involving the reproductive system are encompassed within the scope of

the invention.

- The diagnosis and/or treatment of eye disorders. Examples of such diseases include, but are not limited to, inflammatory eye disease, as may be associated with, for example, corneal transplant; retinal degeneration, blindness, macular degeneration, glaucoma, uveitis, and retinal neuropathy. Other diseases of the eye are encompassed within the scope of the invention.

- The diagnosis and/or treatment of diseases involving inflammation. Examples of such diseases include but are not limited to those described herein.

Other diseases which are treatable using agents within the scope of the invention include acute pancreatitis, chronic fatigue syndrome, fibromyalgia, and Kawasaki's disease (MLNS).

Other diseases associated with undesirable levels of one or more of IL-1, IL-1ra, the ligand of the present IL-17 receptor like polypeptide, and/or the present IL-17 receptor like polypeptide itself are encompassed within the scope of the invention. Undesirable levels include excessive and/or sub-normal levels of IL-1, IL-1ra, the ligand of the present IL-17 receptor like polypeptide, and/or the IL-17 receptor like polypeptides described herein.

As contemplated by the present invention, an agonist or antagonist of the IL-17 receptor like polypeptide (including, but not limited to, anti-IL-17 receptor like selective binding agents (such as antibodies), ligands to the IL-17 receptor like receptor, soluble IL-17 receptor like polypeptides, small molecules, and antisense oligonucleotides, or an IL-17 receptor like polypeptide itself) may be administered as an adjunct to other therapy and also with other pharmaceutical compositions suitable for the indication being treated. An

agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself and any of one or more additional therapies or pharmaceutical formulations may be administered separately, sequentially, or simultaneously.

In a specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more IL-1 inhibitors for the treatment or prevention of the diseases and disorders recited herein..

IL-1 inhibitors include any protein capable of specifically preventing activation of cellular receptors to IL-1, which may result from any number of mechanisms. Such mechanisms include downregulating IL-1 production, binding free IL-1, interfering with IL-1 binding to its receptor, interfering with formation of the IL-1 receptor complex (i.e., association of IL-1 receptor with IL-1 receptor accessory protein), or interfering with modulation of IL-1 signaling after binding to its receptor. Classes of interleukin-1 inhibitors include:

- Interleukin-1 receptor antagonists such as IL-1ra, as described herein;
- Anti-IL-1 receptor monoclonal antibodies (e.g., EP 623674);
- IL-1 binding proteins such as soluble IL-1 receptors (e.g., U.S. Pat. No. 5,492,888, U.S. Pat. No. 5,488,032, and U.S. Pat. No. 5,464,937, U.S. Pat. No. 5,319,071, and U.S. Pat. No. 5,180,812;
- Anti-IL-1 monoclonal antibodies (e.g., WO 9501997, WO

9402627, WO 9006371, U.S. Pat. No. 4,935,343, EP 364778, EP 267611 and EP 220063;

- IL-1 receptor accessory proteins and antibodies thereto (e.g., WO 96/23067);
- 5 • Inhibitors of interleukin-1 β converting enzyme (ICE) or caspase I, which can be used to inhibit IL-1 beta production and secretion;
- Interleukin-1 β protease inhibitors;
- Other compounds and proteins which block *in vivo* synthesis or extracellular release of IL-1.

Exemplary IL-1 inhibitors are disclosed in the following references:

US Pat. Nos. 5747444; 5359032; 5608035; 5843905; 5359032; 5866576; 5869660; 5869315; 5872095; 5955480;

15 International (WO) patent applications 98/21957, 96/09323, 91/17184, 96/40907, 98/32733, 98/42325, 98/44940, 98/47892, 98/56377, 99/03837, 99/06426, 99/06042, 91/17249, 98/32733, 98/17661, 97/08174, 95/34326, 99/36426, and 99/36415;

20 European (EP) patent applications 534978 and 894795; and French patent application FR 2762514;

Interleukin-1 receptor antagonist (IL-1ra) is a human protein that acts as a natural inhibitor of interleukin-1. Preferred receptor antagonists (including IL-1ra and variants and derivatives thereof), as well as methods of making and using thereof, are described in U.S. Patent No. 5,075,222; WO 91/08285; WO 91/17184; AU 9173636; WO 92/16221; WO93/21946; WO 94/06457; WO 94/21275; FR 2706772; WO 94/21235; DE 4219626, WO 94/20517; WO 96/22793; WO 97/28828; and WO 99/36541. The proteins include glycosylated as well as non-glycosylated IL-1

receptor antagonists.

Those skilled in the art will recognize that many combinations of deletions, insertions, and substitutions (individually or collectively "variant(s)" herein) can be made within the amino acid sequences of IL-1ra, provided that the resulting molecule is biologically active (e.g., possesses the ability to affect one or more of the diseases and disorders such as those recited herein.)

In a specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more TNF inhibitors for the treatment or prevention of the diseases and disorders recited herein.

Such TNF inhibitors include compounds and proteins which block *in vivo* synthesis or extracellular release of TNF. In a specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more of the following TNF inhibitors: TNF binding proteins (soluble TNF receptor type-I and soluble TNF receptor type-II ("sTNFRs"), as defined herein), anti-TNF antibodies, granulocyte colony stimulating factor; thalidomide; BN 50730; tenidap; E 5531; tiapafant PCA 4248; nimesulide; panavir; rolipram; RP 73401; peptide T; MDL 201,449A; (1R,3S)-Cis-1-[9-(2,6-diaminopuriny)]-3-hydroxy-4-cyclopentene hydrochloride; (1R,3R)-trans-1-(9-(2,6-diamino)purine]-3-acetoxycyclopentane; (1R,3R)-trans-1-[9-adenyl)-3-azidocyclopentane hydrochloride and (1R,3R)-trans-1-(6-hydroxy-purin-9-yl)-3-azidocyclopentane. TNF binding proteins are disclosed in the art (EP 308 378, EP 422 339, GB

2 218 101, EP 393 438, WO 90/13575, EP 398 327, EP 412 486, WO
91/03553, EP 418 014, JP 127,800/1991, EP 433 900, U.S. Patent
No. 5,136,021, GB 2 246 569, EP 464 533, WO 92/01002,
WO 92/13095, WO 92/16221, EP 512 528, EP 526 905, WO 93/07863,
5 EP 568 928, WO 93/21946, WO 93/19777, EP 417 563, WO 94/06476,
and PCT International Application No. PCT/US97/12244).

For example, EP 393 438 and EP 422 339 teach the amino
acid and nucleic acid sequences of a soluble TNF receptor type
I (also known as "sTNFR-I" or "30kDa TNF inhibitor") and a
10 soluble TNF receptor type II (also known as "sTNFR-II" or
"40kDa TNF inhibitor"), collectively termed "sTNFRs", as well
as modified forms thereof (e.g., fragments, functional
derivatives and variants). EP 393 438 and EP 422 339 also
disclose methods for isolating the genes responsible for
15 coding the inhibitors, cloning the gene in suitable vectors
and cell types and expressing the gene to produce the
inhibitors. Additionally, polyvalent forms (i.e., molecules
comprising more than one active moiety) of sTNFR-I and sTNFR-
II have also been disclosed. In one embodiment, the
20 polyvalent form may be constructed by chemically coupling at
least one TNF inhibitor and another moiety with any clinically
acceptable linker, for example polyethylene glycol
(WO 92/16221 and WO 95/34326), by a peptide linker (Neve et
al. (1996), *Cytokine*, 8(5):365-370, by chemically coupling to
25 biotin and then binding to avidin (WO 91/03553) and, finally,
by combining chimeric antibody molecules (U.S. Patent
5,116,964, WO 89/09622, WO 91/16437 and EP 315062).

Anti-TNF antibodies include MAK 195F Fab antibody (Holler
et al. (1993), 1st International Symposium on Cytokines in
30 Bone Marrow Transplantation, 147); CDP 571 anti-TNF monoclonal
antibody (Rankin et al. (1995), *British Journal of
Rheumatology*, 34:334-342); BAY X 1351 murine anti-tumor
necrosis factor monoclonal antibody (Kieft et al. (1995),

7th European Congress of Clinical Microbiology and Infectious Diseases, page 9); CentTNF cA2 (REMICADE) anti-TNF monoclonal antibody (Elliott et al. (1994), *Lancet*, 344:1125-1127 and Elliott et al. (1994), *Lancet*, 344:1105-1110).

5 It will be appreciated that the IL-17 receptor like polypeptides may be used (simultaneously or sequentially) in combination with one or more cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

10 In one specific embodiment, the present invention is directed to the use of agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with secreted or soluble
5 human fas antigen or recombinant versions thereof (WO 96/20206 and Mountz et al., *J. Immunology*, 155:4829-4837; and EP 510 691. WO 96/20206 discloses secreted human fas antigen (native and recombinant, including an Ig fusion protein), methods for isolating the genes responsible for coding the soluble recombinant human fas antigen, methods for cloning the gene in suitable vectors and cell types, and methods for expressing the gene to produce the inhibitors. EP 510 691 describes DNAs coding for human fas antigen, including soluble fas antigen, vectors expressing for said DNAs and transformants transfected
25 with the vector. When administered parenterally, doses of a secreted or soluble fas antigen fusion protein each are generally from about 1 micrograms/kg to about 100 micrograms/kg.

30 Treatment of the diseases and disorders recited herein, can include the use of first line drugs for control of pain and inflammation. These drugs are classified as non-steroidal, anti-inflammatory drugs (NSAIDs). Secondary treatments include corticosteroids, slow acting antirheumatic

drugs (SAARDs), or disease modifying (DM) drugs. Information regarding the following compounds can be found in The Merck Manual of Diagnosis and Therapy, Sixteenth Edition, Merck, Sharp & Dohme Research Laboratories, Merck & Co., Rahway, NJ (1992) and in Pharmaprojects, PJB Publications Ltd.

In a specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself and any of one or more NSAIDs for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases; and graft versus host disease. NSAIDs owe their anti-inflammatory action, at least in part, to the inhibition of prostaglandin synthesis (Goodman and Gilman in "The Pharmacological Basis of Therapeutics," MacMillan 7th Edition (1985)). NSAIDs can be characterized into at least nine groups: (1) salicylic acid derivatives; (2) propionic acid derivatives; (3) acetic acid derivatives; (4) fenamic acid derivatives; (5) carboxylic acid derivatives; (6) butyric acid derivatives; (7) oxicams; (8) pyrazoles and (9) pyrazolones.

In another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more salicylic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. Such salicylic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: acetaminosalol, aloxiprin, aspirin, benorylate, bromosaligenin, calcium acetylsalicylate, choline magnesium trisalicylate, magnesium salicylate, choline salicylate, diflusal, etersalate, fendosal, gentisic acid, glycol salicylate, imidazole salicylate, lysine acetylsalicylate,

mesalamine, morpholine salicylate, 1-naphthyl salicylate, olsalazine, parsalimide, phenyl acetylsalicylate, phenyl salicylate, salacetamide, salicylamide O-acetic acid, salsalate, sodium salicylate and sulfasalazine. Structurally related salicylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more propionic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The propionic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: alminoprofen, benoxaprofen, bucloxic acid, carprofen, dexindoprofen, fenoprofen, flunoxaprofen, fluprofen, flurbiprofen, furocloprofen, ibuprofen, ibuprofen aluminum, ibuprofen sodium, indoprofen, isoprofen, ketoprofen, loxoprofen, miroprofen, naproxen, naproxen sodium, oxapropazone, piroxicam, piroxicam sodium, pranoprofen, protizinic acid, pyridoxicarboxylic acid, suprofen, tiaprofenic acid and tioxaprofen. Structurally related propionic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In yet another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more acetic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The acetic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof

comprise: acetaminophen, alclometacin, amfenac, bufexamac, cinmetacin, clopirac, delmetacin, diclofenac potassium, diclofenac sodium, etodolac, felbinac, fenclofenac, fenclorac, fenclozic acid, fentiazac, furofenac, glucametacin, ibufenac, 5 indomethacin, isofezolac, isoxepac, lonazolac, metiazinic acid, oxametacin, oxpinac, pimetacin, proglumetacin, sulindac, talmetacin, tiaramide, tiopinac, tolmetin, tolmetin sodium, zidometacin and zomepirac. Structurally related acetic acid derivatives having similar analgesic and anti-inflammatory 10 properties are also intended to be encompassed by this group.

15
20
25
In another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more fenamic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The fenamic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: enfenamic acid, etofenamate, flufenamic acid, 20 isonixin, meclofenamic acid, meclofenamate sodium, medofenamic acid, mefenamic acid, niflumic acid, talniflumate, terofenamate, tolfenamic acid and ufenamate. Structurally related fenamic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be 25 encompassed by this group.

30
In an additional specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more carboxylic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The carboxylic acid derivatives, prodrug esters, and pharmaceutically

acceptable salts thereof which can be used comprise: clidanac, diflunisal, flufenisal, inoridine, ketorolac and tinoridine. Structurally related carboxylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In yet another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more butyric acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The butyric acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: bumadizon, butibufen, fenbufen and xenbucin. Structurally related butyric acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more oxicams, prodrug esters, or pharmaceutically acceptable salts thereof. The oxicams, prodrug esters, and pharmaceutically acceptable salts thereof comprise: droxicam, enolicam, isoxicam, piroxicam, sudoxicam, tenoxicam and 4-hydroxyl-1,2-benzothiazine-1,1-dioxide-4-(N-phenyl)-carboxamide. Structurally related oxicams having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17

receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more pyrazoles, prodrug esters, or pharmaceutically acceptable salts thereof. The pyrazoles, prodrug esters, and pharmaceutically acceptable salts thereof which may be used comprise: difenamizole and epirizole. Structurally related pyrazoles having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment or, concurrent treatment) with any of one or more pyrazolones, prodrug esters, or pharmaceutically acceptable salts thereof. The pyrazolones, prodrug esters and pharmaceutically acceptable salts thereof which may be used comprise: apazone, azapropazone, benzpiperylon, feprazone, mofebutazone, morazone, oxyphenbutazone, phenylbutazone, pipebuzone, propylphenazone, ramifenazone, suxibuzone and thiazolinobutazone. Structurally related pyrazalones having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more of the following NSAIDs: ϵ -acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, anitrazafen, antrafenine, bendazac, bendazac lysinate, benzydamine, beprozoin, broperamole, bucolome, bufezolac, ciproquazone, cloximate, dazidamine, deboxamet, detomidine, difenpiramide, difenpyramide, difisalamine, ditazol,

emorfazone, fanetizole mesylate, fenflumizole, floctafenine, flumizole, flunixin, fluproquazone, fopirtoline, fosfosal, guaimesal, guaiazolene, isonixirn, lefetamine HCl, leflunomide, lofemizole, lotifazole, lysin clonixinate, 5 meseclazone, nabumetone, nictindole, nimesulide, orgotein, orpanoxin, oxaceprol, oxapadol, paranyline, perisoxal, perisoxal citrate, pifoxime, piproxen, pirazolac, pirfenidone, proquazone, proxazole, thielavin B, tiflamizole, timegadine, tolectin, tolpadol, tryptamid and those designated by company 10 code number such as 480156S, AA861, AD1590, AFP802, AFP860, AI77B, AP504, AU8001, BPPC, BW540C, CHINOIN 127, CN100, EB382, EL508, F1044, FK-506, GV3658, ITF182, KCNTEI6090, KME4, LA2851, MR714, MR897, MY309, ONO3144, PR823, PV102, PV108, R830, RS2131, SCR152, SH440, SIR133, SPAS510, SQ27239, ST281, 15 SY6001, TA60, TAI-901 (4-benzoyl-1-indancarboxylic acid), TVX2706, U60257, UR2301 and WY41770. Structurally related NSAIDs having similar analgesic and anti-inflammatory properties to the NSAIDs are also intended to be encompassed by this group.

20 In still another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment or concurrent treatment) with any of one or 25 more corticosteroids, prodrug esters or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, graft versus host disease and multiple sclerosis. Corticosteroids, prodrug 30 esters and pharmaceutically acceptable salts thereof include hydrocortisone and compounds which are derived from hydrocortisone, such as 21-acetoxypregnenolone, alclomerasone, algestone, amcinonide, beclomethasone, betamethasone,

betamethasone valerate, budesonide, chloroprednisone, clobetasol, clobetasol propionate, clobetasone, clobetasone butyrate, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacon, desonide, desoximetasone, 5 dexamethasone, diflorasone, diflucortolone, difluprednate, enoxolone, fluazacort, flucloxonide, flumethasone, flumethasone pivalate, flucinolone acetonide, flunisolide, fluocinonide, fluorocinolone acetonide, fluocortin butyl, flucortolone, flucortolone hexanoate, diflucortolone 10 valerate, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, formocortol, halcinonide, halometasone, halopredone acetate, hydrocortamate, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone phosphate, 15 hydrocortisone 21-sodium succinate, hydrocortisone tebutate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 21-diedryaminoacetate, prednisolone sodium phosphate, prednisolone sodium succinate, prednisolone 20 sodium 21-*m*-sulfobenzoate, prednisolone sodium 21-stearoglycolate, prednisolone tebutate, prednisolone 21-trimethylacetate, prednisone, prednival, prednylidene, prednylidene 21-diethylaminoacetate, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone 25 benetonide and triamcinolone hexacetonide. Structurally related corticosteroids having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is 30 directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more

slow-acting antirheumatic drugs (SAARDs) or disease modifying antirheumatic drugs (DMARDs), prodrug esters, or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, graft versus host disease and multiple sclerosis. SAARDs or DMARDs, prodrug esters and pharmaceutically acceptable salts thereof comprise: allocupreide sodium, auranofin, aurothioglucose, aurothioglycanide, azathioprine, brequinar sodium, bucillamine, calcium 3-aurothio-2-propanol-1-sulfonate, chlorambucil, chloroquine, clobuzarit, cuproxoline, cyclophosphamide, cyclosporin, dapsone, 15-deoxyspergualin, diacerein, glucosamine, gold salts (e.g., cycloquine gold salt, gold sodium thiomalate, gold sodium thiosulfate), hydroxychloroquine, hydroxychloroquine sulfate, hydroxyurea, kebuzone, levamisole, lobenzarit, melittin, 6-mercaptopurine, methotrexate, mizoribine, mycophenolate mofetil, myoral, nitrogen mustard, D-penicillamine, pyridinol imidazoles such as SKNF86002 and SB203580, rapamycin, thiols, thymopoietin and vincristine. Structurally related SAARDs or DMARDs having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more COX2 inhibitors, prodrug esters or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation. Examples of COX2 inhibitors, prodrug esters or pharmaceutically acceptable salts thereof include, for example, celecoxib. Structurally related COX2 inhibitors

having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an agonist or antagonist of the IL-17 receptor like polypeptide, and/or an IL-17 receptor like polypeptide itself in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more antimicrobials, prodrug esters or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation. Antimicrobials include, for example, the broad classes of penicillins, cephalosporins and other beta-lactams, aminoglycosides, azoles, quinolones, macrolides, rifamycins, tetracyclines, sulfonamides, lincosamides and polymyxins. The penicillins include, but are not limited to penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, floxacillin, ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanate, hetacillin, cyclacillin, bacampicillin, carbenicillin, carbenicillin indanyl, ticarcillin, ticarcillin/clavulanate, azlocillin, mezlocillin, peperacillin, and mecillinam. The cephalosporins and other beta-lactams include, but are not limited to cephalothin, cephapirin, cephalixin, cephradine, cefazolin, cefadroxil, cefaclor, cefamandole, cefotetan, cefoxitin, ceruroxime, cefonicid, ceforadine, cefixime, cefotaxime, moxalactam, ceftizoxime, ceftriaxone, cephooperazone, ceftazidime, imipenem and aztreonam. The aminoglycosides include, but are not limited to streptomycin, gentamicin, tobramycin, amikacin, netilmicin, kanamycin and neomycin. The azoles include, but are not limited to fluconazole. The quinolones include, but are not limited to nalidixic acid, norfloxacin, enoxacin, ciprofloxacin, ofloxacin, sparfloxacin and temafloxacin. The macrolides include, but are not limited to erythromycin,

spiramycin and azithromycin. The rifamycins include, but are not limited to rifampin. The tetracyclines include, but are not limited to spicycline, chlortetracycline, clomocycline, demeclocycline, deoxycycline, guamecycline, lymecycline, 5 meclocycline, methacycline, minocycline, oxytetracycline, penimepicycline, pipacycline, rolitetracycline, sancycline, senociclin and tetracycline. The sulfonamides include, but are not limited to sulfanilamide, sulfamethoxazole, sulfacetamide, sulfadiazine, sulfisoxazole and co-trimoxazole 10 (trimethoprim/sulfamethoxazole). The lincosamides include, but are not limited to clindamycin and lincomycin. The polymyxins (polypeptides) include, but are not limited to polymyxin B and colistin.

IL-17 Receptor Like Compositions and Administration

15 Therapeutic compositions are within the scope of the present invention. Such IL-17 receptor like pharmaceutical compositions may comprise a therapeutically effective amount of an IL-17 receptor like polypeptide or an IL-17 receptor like nucleic acid molecule in admixture with a 20 pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Other pharmaceutical compositions may comprise a therapeutically effective amount of one or more IL-17 receptor like selective binding agents in admixture with a 25 pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

30 The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for

example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrans), proteins (such as serum albumin, gelatin or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide), solvents (such as glycerin, propylene glycol or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal), stability enhancing agents (sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride), mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. See *Remington's Pharmaceutical Sciences*, 18th Ed., A.R. Gennaro, ed., Mack Publishing Company (1990).

The optimal pharmaceutical formulation will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See for example, *Remington's Pharmaceutical Sciences*,
5 *supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the IL-17 receptor like molecule.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature.
10 For example, a suitable vehicle or carrier may be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with
15 serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present invention, IL-17 receptor
20 like polypeptide compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized cake or an aqueous solution. Further, the IL-17 receptor like
25 polypeptide product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The IL-17 receptor like pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery
30 through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations

that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

5 When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired IL-17 receptor like molecule in a pharmaceutically acceptable vehicle. A particularly
10 suitable vehicle for parenteral injection is sterile distilled water in which a IL-17 receptor like molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-
15 erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), or beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered as a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting
20 sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

Pharmaceutical compositions such as (1) slow-release formulations, (2) inhalant mists, or (3) orally active
25 formulations are also envisioned. The IL-17 receptor like molecule pharmaceutical composition generally is formulated for parenteral administration. Such parenterally administered therapeutic compositions are typically in the form of a pyrogen-free, parenterally acceptable aqueous solution
30 comprising the desired IL-17 receptor like molecule in a pharmaceutically acceptable vehicle. The IL-17 receptor like molecule pharmaceutical compositions also may include particulate preparations of polymeric compounds such as

polylactic acid, polyglycolic acid, etc. or the introduction of the molecule into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation

5 In one embodiment, a pharmaceutical composition may be formulated for inhalation. For example, an IL-17 receptor like polypeptide may be formulated as a dry powder for inhalation. IL-17 receptor like polypeptide or IL-17 receptor like nucleic acid molecule inhalation solutions may also be
10 formulated with a liquefied propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT application no. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

15 It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, IL-17 receptor like polypeptides which are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid
20 dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate
25 absorption of the IL-17 receptor like molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Another pharmaceutical composition may involve an
30 effective quantity of IL-17 receptor like polypeptides in a mixture with non-toxic excipients which are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared

in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional IL-17 receptor like pharmaceutical formulations will be evident to those skilled in the art, including formulations involving IL-17 receptor like polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 which describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer et al., *J. Biomed. Mater. Res.*, 15:167-277 (1981) and Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art. See e.g., Eppstein et al., *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

The IL-17 receptor like pharmaceutical composition to be used for *in vivo* administration typically must be sterile.

This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution.

5 The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection
10 needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form
15 or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having
20 a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

An effective amount of an IL-17 receptor like
25 pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the
30 indication for which the IL-17 receptor like molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer

the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 $\mu\text{g/kg}$ to up to about 100 mg/kg or more, depending on the factors mentioned above. In other
5 embodiments, the dosage may range from 0.1 $\mu\text{g/kg}$ up to about 100 mg/kg; or 1 $\mu\text{g/kg}$ up to about 100 mg/kg; or 5 $\mu\text{g/kg}$ up to about 100 mg/kg.

The frequency of dosing will depend upon the pharmacokinetic parameters of the IL-17 receptor like molecule
10 in the formulation used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired
15 molecule) over time, or as a continuous infusion via implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained
20 through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, e.g. oral, inhalation, injection or infusion by intravenous, intraperitoneal, intracerebral (intra-parenchymal),
25 intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes, or by sustained release systems or implantation device. Where desired, the compositions may be administered by continuously by infusion, by bolus injection or continuously by infusion,
30 or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation into the affected area

of a membrane, sponge, or other appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be directly through the device implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, time release bolus, or continuous administration, or via catheter continuous infusion.

It will further be appreciated that the IL-17 receptor like polypeptides, including fragments, variants, and derivatives, may be employed alone, together, or in combination with other polypeptides and pharmaceutical compositions. For example, the IL-17 receptor like polypeptides may be used in combination with cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

In some cases, it may be desirable to use IL-17 receptor like pharmaceutical compositions in an ex vivo manner. In such instances, cells, tissues, or organs that have been removed from the patient are exposed to IL-17 receptor like pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, an IL-17 receptor like polypeptide can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation

materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Additional embodiments of the present invention relate to cells and methods (e.g., homologous recombination and/or other recombinant production methods) for both the *in vitro* production of therapeutic polypeptides and for the production and delivery of therapeutic polypeptides by gene therapy or cell therapy. Homologous and other recombination methods may be used to modify a cell that contains a normally transcriptionally silent IL-17 receptor like gene, or an under expressed gene, and thereby produce a cell which expresses therapeutically efficacious amounts of IL-17 receptor like polypeptides.

It is further envisioned that IL-17 receptor like polypeptides can be produced *in vitro* or *in vivo* by homologous recombination, or with recombinant production methods utilizing control elements introduced into cells already containing DNA encoding IL-17 receptor polypeptides. For example, homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes (Kucherlapati, *Prog. in Nucl. Acid Res. & Mol. Biol.*, 36:301, 1989). The basic technique was developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas et al., *Cell*, 44:419-428, 1986; Thomas and Capecchi, *Cell*, 51:503-512, 1987; Doetschman et al., *Proc. Natl. Acad. Sci.*, 85:8583-8587, 1988) or to correct specific mutations within defective genes (Doetschman et al., *Nature*, 330:576-578, 1987). Exemplary homologous recombination

techniques are described in U.S. Patent No. 5,272,071 (EP 9193051, EP Publication No. 505500; PCT/US90/07642, International Publication No. WO 91/09955).

Through homologous recombination, the DNA sequence to be
5 inserted into the genome can be directed to a specific region
of the gene of interest by attaching it to targeting DNA. The
targeting DNA is a nucleotide sequence that is complementary
(homologous) to a region of the genomic DNA. Small pieces of
targeting DNA that are complementary to a specific region of
10 the genome are put in contact with the parental strand during
the DNA replication process. It is a general property of DNA
that has been inserted into a cell to hybridize, and
therefore, recombine with other pieces of endogenous DNA
through shared homologous regions. If this complementary
15 strand is attached to an oligonucleotide that contains a
mutation or a different sequence or an additional nucleotide,
it too is incorporated into the newly synthesized strand as a
result of the recombination. As a result of the proofreading
function, it is possible for the new sequence of DNA to serve
20 as the template. Thus, the transferred DNA is incorporated
into the genome.

Attached to these pieces of targeting DNA are regions of
DNA which may interact with or control the expression of a IL-
17 receptor like polypeptide, e.g., flanking sequences. For
25 example, a promoter/enhancer element, a suppresser, or an
exogenous transcription modulatory element is inserted in the
genome of the intended host cell in proximity and orientation
sufficient to influence the transcription of DNA encoding the
desired IL-17 receptor like polypeptide. The control element
30 controls a portion of the DNA present in the host cell genome.
Thus, the expression of the desired IL-17 receptor like
polypeptide may be achieved not by transfection of DNA that

encodes the IL-17 receptor like gene itself, but rather by the use of targeting DNA (containing regions of homology with the endogenous gene of interest) coupled with DNA regulatory segments that provide the endogenous gene sequence with
5 recognizable signals for transcription of an IL-17 receptor like polypeptide.

In an exemplary method, the expression of a desired targeted gene in a cell (i.e., a desired endogenous cellular gene) is altered via homologous recombination into the
10 cellular genome at a preselected site, by the introduction of DNA which includes at least a regulatory sequence, an exon and a splice donor site. These components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit
15 (in which the regulatory sequence, the exon and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of these components into the chromosomal DNA, the expression of the desired endogenous gene is altered.

20 Altered gene expression, as described herein, encompasses activating (or causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a gene which is not expressed at physiologically significant levels in the cell as obtained.
25 The embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regulation or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

30 One method by which homologous recombination can be used to increase, or cause, IL-17 receptor like polypeptide production from a cell's endogenous IL-17 receptor like gene

involves first using homologous recombination to place a recombination sequence from a site-specific recombination system (e.g., Cre/loxP, FLP/FRT) (Sauer, *Current Opinion In Biotechnology*, 5:521-527, 1994; Sauer, *Methods In Enzymology*, 225:890-900, 1993) upstream (that is, 5' to) of the cell's endogenous genomic IL-17 receptor like polypeptide coding region. A plasmid containing a recombination site homologous to the site that was placed just upstream of the genomic IL-17 receptor like polypeptide coding region is introduced into the modified cell line along with the appropriate recombinase enzyme. This recombinase causes the plasmid to integrate, via the plasmid's recombination site, into the recombination site located just upstream of the genomic IL-17 receptor like polypeptide coding region in the cell line (Baubonis and Sauer, *Nucleic Acids Res.*, 21:2025-2029, 1993; O'Gorman et al., *Science*, 251:1351-1355, 1991). Any flanking sequences known to increase transcription (e.g., enhancer/promoter, intron, translational enhancer), if properly positioned in this plasmid, would integrate in such a manner as to create a new or modified transcriptional unit resulting in *de novo* or increased IL-17 receptor like polypeptide production from the cell's endogenous IL-17 receptor like gene.

A further method to use the cell line in which the site specific recombination sequence had been placed just upstream of the cell's endogenous genomic IL-17 receptor like polypeptide coding region is to use homologous recombination to introduce a second recombination site elsewhere in the cell line's genome. The appropriate recombinase enzyme is then introduced into the two-recombination-site cell line, causing a recombination event (deletion, inversion, translocation) (Sauer, *Current Opinion In Biotechnology*, *supra*, 1994; Sauer, *Methods In Enzymology*, *supra*, 1993) that would create a new or modified transcriptional unit resulting in *de novo* or

increased IL-17 receptor like polypeptide production from the cell's endogenous IL-17 receptor like gene.

An additional approach for increasing, or causing, the expression of IL-17 receptor like polypeptide from a cell's endogenous IL-17 receptor like gene involves increasing, or causing, the expression of a gene or genes (e.g., transcription factors) and/or decreasing the expression of a gene or genes (e.g., transcriptional repressors) in a manner which results in *de novo* or increased IL-17 receptor like polypeptide production from the cell's endogenous IL-17 receptor like gene. This method includes the introduction of a non-naturally occurring polypeptide (e.g., a polypeptide comprising a site specific DNA binding domain fused to a transcriptional factor domain) into the cell such that *de novo* or increased IL-17 receptor like polypeptide production from the cell's endogenous IL-17 receptor like gene results.

The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. The targeting sequence in the DNA construct directs the integration of elements (a)-(d) into a target gene in a cell such that the elements (b)-(d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)-(f) such that the elements of (b)-(f) are operatively linked to the endogenous gene. The targeting sequence is homologous to the preselected site in

the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon.

5 If the sequence of a particular gene is known, such as the nucleic acid sequence encoding an IL-17 receptor like polypeptide presented herein, a piece of DNA that is complementary to a selected region of the gene can be synthesized or otherwise obtained, such as by appropriate
10 restriction of the native DNA at specific recognition sites bounding the region of interest. This piece serves as a targeting sequence(s) upon insertion into the cell and will hybridize to its homologous region within the genome. If this hybridization occurs during DNA replication, this piece of
15 DNA, and any additional sequence attached thereto, will act as an Okazaki fragment and will be incorporated into the newly synthesized daughter strand of DNA. The present invention, therefore, includes nucleotides encoding a IL-17 receptor like polypeptide, which nucleotides may be used as targeting
20 sequences.

IL-17 receptor like polypeptide cell therapy, e.g., the implantation of cells producing IL-17 receptor like polypeptides, is also contemplated. This embodiment involves implanting cells capable of synthesizing and secreting a
25 biologically active form of IL-17 receptor like polypeptide. Such IL-17 receptor like polypeptide-producing cells can be cells that are natural producers of IL-17 receptor like polypeptides or may be recombinant cells whose ability to produce IL-17 receptor like polypeptides has been augmented by
30 transformation with a gene encoding the desired IL-17 receptor like polypeptide or with a gene augmenting the expression of IL-17 receptor like polypeptide. Such a modification may be

accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered an IL-17 receptor like polypeptide, as may occur with the administration of a polypeptide of a foreign species, it is preferred that the natural cells producing IL-17 receptor like polypeptide be of human origin and produce human IL-17 receptor like polypeptide. Likewise, it is preferred that the recombinant cells producing IL-17 receptor like polypeptide be transformed with an expression vector containing a gene encoding a human IL-17 receptor like polypeptide.

Implanted cells may be encapsulated to avoid the infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in biocompatible, semipermeable polymeric enclosures or membranes that allow the release of IL-17 receptor like polypeptide, but that prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce IL-17 receptor like polypeptides *ex vivo*, may be implanted directly into the patient without such encapsulation.

Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be routinely accomplished. For example, Baetge et al. (WO95/05452; PCT/US94/09299) describe membrane capsules containing genetically engineered cells for the effective delivery of biologically active molecules. The capsules are biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively

linked to promoters that are not subject to down regulation in vivo upon implantation into a mammalian host. The devices provide for the delivery of the molecules from living cells to specific sites within a recipient. In addition, see U.S. Patent Nos. 4,892,538, 5,011,472, and 5,106,627. A system for encapsulating living cells is described in PCT Application no. PCT/US91/00157 of Aebischer et al. See also, PCT Application no. PCT/US91/00155 of Aebischer et al., Winn et al., *Exper. Neurol.*, 113:322-329 (1991), Aebischer et al., *Exper. Neurol.*, 111:269-275 (1991); and Tresco et al., *ASAIO*, 38:17-23 (1992).

In vivo and in vitro gene therapy delivery of IL-17 receptor like polypeptides is also envisioned. One example of a gene therapy technique is to use the IL-17 receptor like gene (either genomic DNA, cDNA, and/or synthetic DNA) encoding a IL-17 receptor like polypeptide which may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct". The promoter may be homologous or heterologous to the endogenous IL-17 receptor like gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include, DNA molecules designed for site-specific integration (e.g., endogenous sequences useful for homologous recombination), tissue-specific promoter, enhancer(s) or silencer(s), DNA molecules capable of providing a selective advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (as, for example, for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as factors to enable vector manufacture.

A gene therapy DNA construct can then be introduced into

cells (either *ex vivo* or *in vivo*) using viral or non-viral vectors. One means for introducing the gene therapy DNA construct is by means of viral vectors as described herein. Certain vectors, such as retroviral vectors, will deliver the DNA construct to the chromosomal DNA of the cells, and the gene can integrate into the chromosomal DNA. Other vectors will function as episomes, and the gene therapy DNA construct will remain in the cytoplasm.

In yet other embodiments, regulatory elements can be included for the controlled expression of the IL-17 receptor like gene in the target cell. Such elements are turned on in response to an appropriate effector. In this way, a therapeutic polypeptide can be expressed when desired. One conventional control means involves the use of small molecule dimerizers or rapalogs (as described in WO9641865 (PCT/US96/099486); WO9731898 (PCT/US97/03137) and WO9731899 (PCT/US95/03157) used to dimerize chimeric proteins which contain a small molecule-binding domain and a domain capable of initiating biological process, such as a DNA-binding protein or transcriptional activation protein. The dimerization of the proteins can be used to initiate transcription of the transgene.

An alternative regulation technology uses a method of storing proteins expressed from the gene of interest inside the cell as an aggregate or cluster. The gene of interest is expressed as a fusion protein that includes a conditional aggregation domain which results in the retention of the aggregated protein in the endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can be released, however, by administering a drug (e.g., small molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the

aggregates or clusters so that the proteins may be secreted from the cell. See, *Science* 287:816-817, and 826-830 (2000).

Other suitable control means or gene switches include, but are not limited to, the following systems. Mifepristone (RU486) is used as a progesterone antagonist. The binding of a modified progesterone receptor ligand-binding domain to the progesterone antagonist activates transcription by forming a dimer of two transcription factors which then pass into the nucleus to bind DNA. The ligand binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further described in U.S. 5,364,791; WO9640911, and WO9710337.

Yet another control system uses ecdysone (a fruit fly steroid hormone) which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation domain/DNA-binding domain/ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. 5,514,578; WO9738117; WO9637609; and WO9303162.

Another control means uses a positive tetracycline-controllable transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated tet R-4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, i.e., it binds to a tet operator in the presence of tetracycline) linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Nos. 5,464,758; 5,650,298 and 5,654,168.

Additional expression control systems and nucleic acid

constructs are described in U.S. Patent Nos. 5,741,679 and 5,834,186, to Innovir Laboratories Inc.

In vivo gene therapy may be accomplished by introducing the gene encoding an IL-17 receptor like polypeptide into cells via local injection of an IL-17 receptor like nucleic acid molecule or by other appropriate viral or non-viral delivery vectors. Hefti, *Neurobiology*, 25:1418-1435 (1994). For example, a nucleic acid molecule encoding an IL-17 receptor like polypeptide may be contained in an adeno-associated virus (AAV) vector for delivery to the targeted cells (e.g., Johnson, International Publication No. WO95/34670; International Application No. PCT/US95/07178). The recombinant AAV genome typically contains AAV inverted terminal repeats flanking a DNA sequence encoding an IL-17 receptor like polypeptide operably linked to functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not limited to, retrovirus, adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 5,399,346 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells which have been treated *in vitro* to insert a DNA segment encoding a therapeutic protein. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 5,631,236 involving adenoviral vectors; U.S. Patent No. 5,672,510 involving retroviral vectors; and U.S. 5,635,399 involving retroviral vectors expressing cytokines.

Nonviral delivery methods include, but are not limited to, liposome-mediated transfer, naked DNA delivery (direct injection), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (e.g., gene gun). Gene therapy materials and methods may also include the use of inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to identify transformed cells, negative selection systems and expression control systems (safety measures), cell-specific binding agents (for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 4,970,154 involving electroporation techniques; WO96/40958 involving nuclear ligands; U.S. Patent No. 5,679,559 describing a lipoprotein-containing system for gene delivery; U.S. Patent No. 5,676,954 involving liposome carriers; U.S. Patent No. 5,593,875 concerning methods for calcium phosphate transfection; and U.S. Patent No. 4,945,050 wherein biologically active particles are propelled at cells at a speed whereby the particles penetrate the surface of the cells and become incorporated into the interior of the cells.

It is also contemplated that IL-17 receptor like gene therapy or cell therapy can further include the delivery of one or more additional polypeptide(s) in the same or a different cell(s). Such cells may be separately introduced into the patient, or the cells may be contained in a single implantable device, such as the encapsulating membrane described above, or the cells may be separately modified by means of viral vectors.

A means to increase endogenous IL-17 receptor like polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the IL-17 receptor like polypeptide promoter, where the enhancer element(s) can serve to increase transcriptional activity of the IL-17 receptor like gene. The enhancer element(s) used will be selected based on the tissue in which one desires to activate the gene(s); enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a gene encoding a IL-17 receptor like polypeptide is to be "turned on" in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing the IL-17 receptor like polypeptide promoter (and optionally, inserted into a vector and/or 5' and/or 3' flanking sequence(s), etc.) using standard cloning techniques. This construct, known as a "homologous recombination construct", can then be introduced into the desired cells either *ex vivo* or *in vivo*.

Gene therapy also can be used to decrease IL-17 receptor like polypeptide expression by modifying the nucleotide sequence of the endogenous promoter(s). Such modification is typically accomplished via homologous recombination methods. For example, a DNA molecule containing all or a portion of the promoter of the IL-17 receptor like gene(s) selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. For example the TATA box and/or the binding site of a transcriptional activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit promoter activity thereby repressing the transcription of the corresponding IL-17 receptor like gene. The deletion of the TATA box or the transcription activator binding site in the promoter may be accomplished by generating a DNA construct

comprising all or the relevant portion of the IL-17 receptor like polypeptide promoter(s) (from the same or a related species as the IL-17 receptor like gene(s) to be regulated) in which one or more of the TATA box and/or transcriptional
5 activator binding site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. The construct will typically contain at least about
10 500 bases of DNA that correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified. The construct may be introduced into the appropriate cells (either *ex vivo* or *in vivo*) either directly or via a viral vector as described herein. Typically, the
15 integration of the construct into the genomic DNA of the cells will be via homologous recombination, where the 5' and 3' DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

20
Additional Uses of IL-17 receptor like Nucleic Acids and Polypeptides

Nucleic acid molecules of the present invention (including those that do not themselves encode biologically
25 active polypeptides) may be used to map the locations of the IL-17 receptor like gene and related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and *in situ* hybridization.

IL-17 receptor like nucleic acid molecules (including
30 those that do not themselves encode biologically active polypeptides), may be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of an IL-17 receptor like DNA

or corresponding RNA in mammalian tissue or bodily fluid samples.

Other methods may also be employed where it is desirable to inhibit the activity of one or more IL-17 receptor like polypeptides. Such inhibition may be effected by nucleic acid molecules which are complementary to and hybridize to expression control sequences (triple helix formation) or to IL-17 receptor like mRNA. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of the selected IL-17 receptor like gene(s) can be introduced into the cell. Antisense probes may be designed by available techniques using the sequence of IL-17 receptor like polypeptide disclosed herein. Typically, each such antisense molecule will be complementary to the start site (5' end) of each selected IL-17 receptor like gene. When the antisense molecule then hybridizes to the corresponding IL-17 receptor like mRNA, translation of this mRNA is prevented or reduced. Antisense inhibitors provide information relating to the decrease or absence of an IL-17 receptor like polypeptide in a cell or organism.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more IL-17 receptor like polypeptides. In this situation, the DNA encoding a mutant polypeptide of each selected IL-17 receptor like polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

In addition, an IL-17 receptor like polypeptide, whether biologically active or not, may be used as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised. Selective binding agents that bind to an IL-17 receptor like polypeptide (as described herein)

may be used for *in vivo* and *in vitro* diagnostic purposes, including, but not limited to, use in labeled form to detect the presence of IL-17 receptor like polypeptide in a body fluid or cell sample. The antibodies may also be used to
5 prevent, treat, or diagnose a number of diseases and disorders, including those recited herein. The antibodies may bind to an IL-17 receptor like polypeptide so as to diminish or block at least one activity characteristic of an IL-17 receptor like polypeptide, or may bind to a polypeptide to
10 increase at least one activity characteristic of an IL-17 receptor like polypeptide (including by increasing the pharmacokinetics of the IL-17 receptor like polypeptide).

2025-04-22 14:22:00

Example 1

Cloning of a First IL-17 Receptor Like Polypeptide

A 477 base pair EST sequence (termed "zhgb-aa287951"), was identified from the Amgen database. The 1392 bp full-length nucleotide sequence of zhgb-aa287951 was then determined. PCR was performed for screening a plurality of human cDNA libraries. The human cDNA libraries were prepared as follows: total RNA was extracted from various human tissues using standard RNA extraction procedures and poly-A⁺ RNA was selected from this total RNA using standard procedures known to those skilled in the art. Random primed or oligo(dT) primed cDNA was synthesized from this poly-A⁺ RNA using the procedure in the manual of the Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (Gibco-BRL, Inc., Rockville, MD) or using other suitable procedures known to those skilled in the art. The resulting cDNA was digested with appropriate restriction enzymes to create sticky ends to assist in ligation to a cloning vector. This digested cDNA was ligated into the pSPORT 1 cloning vector, or another suitable cloning vector known to those skilled in the art, that had been pre-digested with appropriate restriction enzymes. The ligation products were transformed into *E. coli* using standard techniques known in the art, and transformants were selected on bacterial media plates containing either ampicillin, tetracycline, kanamycin or chloramphenicol, depending upon the specific cloning vector used. The cDNA library consisted of all, or a subset, of these transformants.

Both 5'- and 3'- end RACE was carried out using plasmid DNAs prepared from positive libraries as template using a touchdown protocol as follows. Briefly, the PCR conditions were as follows: 94° for 30 seconds; 5 cycles of 94° for 5 seconds and 72° for 2 minutes; 5 cycles of 94° for 5 seconds and 70° for 2 minutes; 25 cycles of 94° for 5 seconds and 68° for 2 minutes 30 seconds; followed by a final extension of 72° for 7 minutes and 4° hold. This reaction used 50ng of each cDNA library,

10pmol of each primer, 200µM dNTP's (final concentration), and a 1x concentration of Clontech's Advantage-HF2 cDNA Polymerase Mix (Cat# K1914-1) in a 50µl final volume. A nested PCR reaction was done on the primary RACE products. The final PCR products were then subcloned and their nucleotide sequences determined. The PCR-derived DNA fragments were used as probes for screening cDNA libraries and cloning of the cDNA for this gene. PCR was used for both 5' RACE and 3' RACE reactions on the seven positive libraries using a touchdown protocol. The 5' RACE primers used gene specific primer 2429-59 (5' GCA GAC ACT GAG AGC ATT GTA ATC-3'; SEQ ID NO: 8) and a library vector (pCMV-SPORT) primer 1916-83 (5'-GGC TCG TAT GTT GTG TGG AAT TGT GAG CG-3'; SEQ ID NO: 9). The 3' RACE primers used gene specific primer 2429-56 (5'-AGG ATC AAA ACT TTC TTT TCT AC-3'; SEQ ID NO: 10) and a library vector primer 1918-80 (5'-TGC AAG GCG ATT AAG TTG GGT AAC GCC AG-3'; SEQ ID NO: 11).

The PCR conditions were the same as described above. A nested PCR reaction was done on the above sample using 5µl of a 1:50 dilution of the first round PCR 5' and 3' RACE products, 10 pmol each of a nested gene specific primer and a nested vector primer. For 5'-nested RACE the gene specific and vector primers were 5'-GCC GAC GGG GAC GTG GAT GAA C-3' (SEQ ID NO: 12) and 5'-CAT GAT TAC GCC AAG CTC TAA TAC GAC TC-3' (SEQ ID NO: 13), respectively. For the 3'-nested RACE the primers were 5'-CTT CGC CGA GTG CCT GTG CAG-3' (SEQ ID NO: 14) and 5'-TCA CGA CGT TGT AAA ACG ACG GCC AGT G-3' (SEQ ID NO: 15), respectively). The remaining reagents and PCR reaction protocol were identical to those used for the primary RACE reactions.

Ten microliters of the final product from the nested RACE was run on a 1% TBE agarose gel at 5V/cm. Well defined single

bands were isolated from the gel and purified using the Qiagen gel extraction kit (Cat#28704) and submitted for sequencing.

EXAMPLE 2

Cloning of Second and Third IL-17 Receptor Like Isoforms

5 For cloning of the second and the third isoforms of the IL-17 receptor like molecule, two new gene specific primers were used for further PCR reaction on the positive libraries. These primers were as follows: 2469-50 (5'-GCG ATG TCG CTC GTG CTG CTA AG-3'; SEQ ID NO: 16) and 2469-54 (5'-GCA GCC TGG TGA GGT GAA ATT CAC-3'; SEQ ID NO: 17). The PCR conditions used
10 in these reactions were as follows: 94° for 2 minutes; 35 cycles of 94° for 30 seconds, 66° for 30 seconds and 72° for 45 seconds, followed by a final extension of 72° for 7 minutes and 4° hold. This reaction used 50ng of each cDNA library, 10pmol
15 of each primer, 200µM dNTP's (final concentration), and a 1x concentration of Clontech's Advantage-HF2 cDNA Polymerase Mix (Cat# K1914-1) in a 50µl final volume.

20 Ten microliters of the product was run on a 1% TBE agarose gel at 5V/cm. Well defined single bands were isolated from the gel and purified using the Qiagen gel extraction kit (Cat#28704) and submitted for sequencing.

EXAMPLE 3

Presence and Distribution of mRNA in Different Tissues

25 PCR was used to screen a panel of 77 human tissue libraries prepared by using 2.5 pmol each of primers 2429-56 and 2429-59 and 50ng library CDNA (Ready-to-go PCR Beads Amersham Pharmacia Biotech Cat#27-9553). PCR Conditions were 94° for 2 minutes; followed by 35 cycles of 94° for 30 seconds;
30 66° For 30 seconds; 72° for 45 seconds; final extension of 72° for 7 minutes and 4° hold. A 440bp band was identified in 40 sources with varying signal intensity. The results were as

follows:

<u>Tissue Source</u>	<u>Level of expression</u>
Fetal pancrease	+++
Fetal scalp	+++
Fetal gall bladder	+++
Cerebellum	+++
Pons / medulla	+++
(midbrain)LNV block 10	+++
lymphoma cell lines	+++
Prostate tumor T1175	+++
Prostate tumor T1940	+++
Colon tumor T24	+++
Ovary tumor T22	+++
Colon tumor T25	+++
Fetal stomach	++
Fetal bladder	++
Fetal kidney	++
Fetal liver	++
Fetal ovary	++
Fetal femur	++
Fetal calveria	++
Fetal mesentery	++
Fetal spleen	++
Fetal lung	++
Fetal heart	++
Forebrain	++
Testis	++
Trachea	++
Bone(limb)	++
Spinal column	++
Thalamus	++
(midbrain) LNV block10	++
Breast tumor T1485	++
Breast tumor T1543	++
Ovary tumor T23	++
Lung tumor T27	++
Adult T-cells	++
Cytoplasmic breast-	
Carcinoma cell lines	++
Fetal thymus	+
Placenta	+
Uterus	+
Normalized Fetal tissues	+

EXAMPLE 4

Production of IL-17 receptor like polypeptides

A. Bacterial Expression

PCR is used to amplify template DNA sequences encoding a IL-17 receptor like polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary vector, such as pAMG21 (ATCC No. 98113) containing the lux promoter and a gene encoding kanamycin resistance is digested with BamHI and NdeI for directional cloning of inserted DNA. The ligated mixture is transformed into an E. coli host strain by electroporation and transformants are selected for kanamycin resistance. Plasmid DNA from selected colonies is isolated and subjected to DNA sequencing to confirm the presence of the insert.

Transformed host cells are incubated in 2xYT medium containing 30 g/ml kanamycin at 30oC prior to induction. Gene expression is induced by the addition of N-(3-oxohexanoyl)-dl-homoserine lactone to a final concentration of 30 ng/ml followed by incubation at either 30oC or 37oC for six hours. The expression of IL-17 receptor like polypeptide is evaluated by centrifugation of the culture, resuspension and lysis of the bacterial pellets, and analysis of host cell proteins by SDS-polyacrylamide gel electrophoresis.

Inclusion bodies containing IL-17 receptor like polypeptide are purified as follows. Bacterial cells are pelleted by centrifugation and resuspended in water. The cell suspension is lysed by sonication and pelleted by centrifugation at 195,000xg for 5 to 10 minutes. The supernatant is discarded, and the pellet is washed and

transferred to a homogenizer. The pellet is homogenized in 5 ml of a Percoll solution (75% liquid Percoll. 0.15M NaCl) until uniformly suspended and then diluted and centrifuged at 21,600xg for 30 minutes. Gradient fractions containing the inclusion bodies are recovered and pooled. The isolated inclusion bodies are analyzed by SDS-PAGE.

A single band on an SDS polyacrylamide gel corresponding to E. coli produced IL-17 receptor like polypeptide is excised from the gel, and the N-terminal amino acid sequence is determined essentially as described by Matsudaira et al., J. Biol. Chem., 262:10-35 (1987).

B. Mammalian Cell Production

PCR is used to amplify template DNA sequences encoding a IL-17 receptor like polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The primer sequences corresponding to the 5' and 3' ends are described above. The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary expression vector, pCEP4 (Invitrogen, Carlsbad, CA), which contains an Epstein-Barr virus origin of replication, may be used for the expression of IL-17 receptor like in 293-EBNA-1 (Epstein-Barr virus nuclear antigen) cells. Amplified and gel purified PCR products are ligated into pCEP4 vector and lipofected into 293-EBNA cells. The transfected cells are selected in 100 g/ml hygromycin and the resulting drug-resistant cultures are grown to confluence. The cells are then cultured in serum-free media for 72 hours. The conditioned media is removed and, IL-17 receptor like polypeptide expression is analyzed by SDS-PAGE.

IL-17 receptor like polypeptide expression may be detected by silver staining. Alternatively, IL-17 receptor like polypeptide is produced as a fusion protein with an epitope tag, such as an IgG constant domain or a FLAG epitope, which may be detected by Western blot analysis using antibodies to the tag peptide.

IL-17 receptor like polypeptides may be excised from an SDS-polyacrylamide gel, or IL-17 receptor like fusion proteins are purified by affinity chromatography to the epitope tag, and subjected to N-terminal amino acid sequence analysis as described herein.

EXAMPLE 5

Production of Anti-IL-17 receptor like Polypeptide Antibodies

Antibodies to IL-17 receptor like polypeptides may be obtained by immunization with purified protein or with IL-17 receptor like peptides produced by biological or chemical synthesis. Suitable procedures for generating antibodies include those described in Hudson and Hay, Practical Immunology, 2nd Edition, Blackwell Scientific Publications (1980).

In one procedure for the production of antibodies, animals (typically mice or rabbits) are injected with a IL-17 receptor like antigen (such as a IL-17 receptor like polypeptide), and those with sufficient serum titer levels as determined by ELISA are selected for hybridoma production. Spleens of immunized animals are collected and prepared as single cell suspensions from which splenocytes are recovered. The splenocytes are fused to mouse myeloma cells (such as Sp2/0-Ag14 cells; ATCC no. CRL-1581), allowed to incubate in DMEM with 200 U/ml penicillin, 200 g/ml streptomycin sulfate, and 4 mM glutamine, then incubated in HAT selection medium (Hypoxanthine; Aminopterin; Thymidine). After selection, the

tissue culture supernatants are taken from each well containing a hybridoma and tested for anti-IL-17 receptor like antibody production by ELISA.

Alternative procedures for obtaining anti-IL-17 receptor like antibodies may also be employed, such as the immunization of transgenic mice harboring human Ig loci for the production of human antibodies, and the screening of synthetic antibody libraries, such as those generated by mutagenesis of an antibody variable domain.

EXAMPLE 6

Recombinant Human IL-17 receptor like-Fc Fusion Protein:

To prepare IL-17 receptor like-Fc fusion proteins, the extra-cellular domain of the human IL-17 receptor like polypeptide (amino acid #1-292 for IL-17RB-2, amino acid #1-350 for IL-17RB-3, SEQ ID NOS: 2 and 5 respectively) was fused to the human IgG1 heavy chain constant region (Fc). The DNA fragment encoding the human Fc (amino acid sequence set out in SEQ ID NO: 21) with a NotI restriction site at its 5' end and XhoI restriction site at its 3' end were directionally ligated into pCEP4 vector using NotI and XhoI sites. The resulting vector containing the Fc coding sequence in pCEP4 is referred to as pCEP4-Fc vector. DNA fragments encoding the extra-cellular domain of the human IL-17RB-2 or IL-17RB-3 (SEQ ID NOS: 2 and 5 respectively), with an Hind III restriction site and kozak sequence (CCACC) at their 5' end and a NotI restriction site at their 3' end, were generated by PCR. These DNA fragments were directionally ligated into the pCEP4-Fc expression vector using the Hind III and NotI restriction sites and were denoted as pCEP4-huIL-17RB-2 like-Fc or pCEP4-huIL-17RB-3 like-Fc. The integrity of the DNA and the junction sites were confirmed by DNA sequencing using standard methods known in the art.

7/25/01
The pCEP4-huIL-17RB-2 like-Fc plasmid or pCEP4-huIL-17RB-3 like-Fc plasmid (also denoted HIL-17RB-2-Fc and HIL17RB-3-Fc, respectively, and deposited on March 14, 2001 with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110, U.S.A. under Accession Nos. _____ and _____, respectively) were transiently transfected into human 293/EBNA cells using Superfect (Qiagen) according to the manufacturer's instructions. The serum-free conditioned media was harvested from the cells 72 hours after transfection. The recombinant human IL-17RB like-Fc fusion proteins, predicted to have the amino acid sequence APS located at the amino-terminus of the mature protein, were isolated by affinity chromatography using a HiTrap Protein G column (Amersham Pharmacia). The amino acid sequences of the resulting fusion proteins are set out in Figure 22 (IL-17RB-2- Fc fusion protein; SEQ ID NO: 24) and Figure 23 (IL-17RB-3-Fc fusion protein; SEQ ID NO: 25).

The recombinant human IL-17RB like-Fc fusion proteins were dialyzed against PBS buffer for 72 hours at 4°C using Spectra/Pore Membrane MWCO 10,000 (Spectrum Laboratories). Subsequently, the recombinant human IL-17RB like-Fc fusion proteins were electrophoresed on a 10% acrylamide gel (Novex) and stained with Coomassie-Blue. The stained gel was scanned with a densitometer to determine the percent representation of the protein band of interest. Modified Lowry Protein Assay Reagent (Pierce) was used to determine the total protein concentration according to the manufacturer's instructions. Then, the amount of human IL-17 receptor like-Fc fusion protein were calculated by multiplying the percentage of IL-17RB like-Fc fusion proteins by the total protein concentration.

The IL-17RB fusion proteins can also be generated with an Epogen signal peptide (MGVHECPAWLWLLLSLLSLPLGLPVLG (SEQ ID NO: 20) fused in frame into the predicted mature protein instead

of fusing to the native extra-cellular domain as described above.

5

EXAMPLE 7

Recombinant Human IL-17E-Fc Fusion Protein

IL-17E was cloned as described in Example 1 of co-owned, concurrently filed U.S. Patent Application Serial No. _____ entitled "IL-17 like Molecules and Uses Thereof" (attorney docket no. 01017/37128A), hereby incorporated by reference in its entirety. An Epogen signal peptide (EpoSP) fused in frame to the predicted mature protein of the human IL-17E (SEQ ID NO: 23) that was fused in frame to the IgG1 heavy chain constant region (Fc) was engineered as follows to make recombinant mature human IL-17E-Fc fusion protein. The EpoSP DNA encoding for the amino acid sequence MGVECPAWLWLLLSLLSLPLGLPVIG (SEQ ID NO: 20) was inserted into the pCEP4 expression vector (Invitrogen) in between a consensus Kozak sequence (CCACC) at its 5' end and an AscI site at its 3' end. In addition, the Fc DNA fragment encoding for the amino acid sequence set out in SEQ ID NO: 12 and a NotI restriction site at the 5' end of the sequence was inserted at the 3' end of the EpoSP (SEQ ID NO: 20). A thymidine was inserted immediately after the NotI restriction site in order to keep the coding frame the same. The resulting vector containing the EpoSP and the Fc in pCEP4 is referred to as pCEP4-EpoSP-Fc vector.

A DNA fragment, containing an AscI restriction site at the 5' end and a NotI restriction site at the 3' end, coding for the mature human IL-17E protein (SEQ ID NO: 23) without the stop codon was generated by PCR. The mature human IL-17E protein starts at amino acid number 17 (aa17) with the starting methionine as amino acid number one. The AscI site,

which contains a thymidine, was inserted immediately before the codon containing aal7 in order to keep the coding frame the same. The human IL-17E fragment was directionally ligated into the pCEP4-EpoSP-Fc expression vector using the AscI and NotI restriction sites and was denoted as pCEP4-EpoSP-huIL-17E-Fc. The integrity of the DNA and the junction sites were confirmed by DNA sequencing using standard methods known in the art.

The pCEP4-EpoSP-IL-17E-Fc plasmid was transiently transfected into human 293/EBNA cells using Superfect (Qiagen) according to the manufacturer's instructions. The serum-free conditioned media was harvested from the cells 72 hours after transfection. The recombinant human IL-17E -Fc fusion protein, predicted to have the amino acid sequence APS located at the amino-terminus of the mature protein, was isolated by affinity chromatography using a HiTrap Protein G column (Amersham Pharmacia). The recombinant human IL-17E -Fc fusion protein was then dialyzed against PBS buffer for 72 hours at 4°C using Spectra/Pore Membrane MWCO 10,000 (Spectrum Laboratories). Subsequently, the recombinant human IL-17E-Fc fusion protein was electrophoresed on a 10% acrylamide gel (Novex) and stained with Coomassie-Blue. The stained gel was scanned with a densitometer to determine the percent representation of the protein band of interest. Modified Lowry Protein Assay Reagent (Pierce) was used to determine the total protein concentration according to the manufacturer's instructions. Then, the amount of human IL-17E -Fc fusion protein was calculated by multiplying the percentage of IL-17E -Fc fusion protein by the total protein concentration.

EXAMPLE 8

IL-17E Polypeptide Binds to the IL-17 Receptor like

Polypeptides

7237
5 To determine if IL-17E polypeptide (SEQ ID NO: 23) is a
ligand for the IL-17 receptor like polypeptides (SEQ ID NO: 2
and/or 5; IL-17RB-2 and/or IL-17RB-3 respectively),
competitive binding assays were performed with the human B-
lymphoblast cell line GM3104A which has been shown to express
IL-17 receptor like polypeptide by Northern Blot and RT-PCR
10 analyses. The conditioned media from 293E cells transfected
to express IL-17E-Fc fusion protein (SEQ ID NO: 23), prepared
as described in Example 7 above, was collected, concentrated
and used for the binding assay. Specificity of ligand binding
was determined by competition with soluble blocking receptors,
15 either IL-17RB-2-Fc or IL-17RB-3-Fc fusion proteins (SEQ ID
NOS: 22 or 23, respectively). IL-17R-Fc fusion protein
(consisting of the extracellular domain of SEQ ID NO: 3) was
purified from conditioned media collected from transfected
293E cells and used as a control. Conditioned media from 293E
20 cells transfected with IL-17RB-2-Fc or IL-17-RB-3-Fc
(deposited with the ATCC on March 14, 2001 under Accession
Nos. _____ and _____ respectively), as described in Example 6
above, was concentrated (5x) with an Amicon 3Kd cut-off
Centracon (#4203) and used for blocking.

25 Prior to the binding assay, 0.5 ml of IL-17E-Fc fusion
protein containing (1x) conditioned media was added into vials
each containing 0.5 ml 5x conditioned media of IL-17RB-2-Fc,
IL-17RB-3-Fc, or 0.5 ml of 5 µg/ml IL-17R-Fc protein in RPMI
1640. Each vial was incubated on ice for 2 hours.

30 Subsequently, GM3104A cells (1×10^6 cells per sample) were
incubated with 1 ml of 8% FBS/PBS, at 4°C for 1 hour. The
cells were then washed with 0.5% BSA/PBS and incubated with 1
ml of untransfected 293E cell conditioned media, conditioned

media containing IL-17E-Fc or conditioned media containing IL-17E-Fc pre-incubated with blocking receptor (IL-17RB-2 or IL-17RB-3) for 2 hours at 4°C with gentle shaking. After the incubation, the cells were washed 3 times with 1 ml of ice-cold 0.5% BSA/PBS.

Each cell sample was stained with 2µg/100µl goat anti human IgG-Fc-FITC (Chemicon, AP113F) diluted in 0.5% BSA/PBS. The cells were incubated on ice for 1 hour and washed 3 times with 1 ml of ice-cold 0.5% BSA/PBS. Subsequently, ligand binding was detected with fluorescence-activated cell sorter analysis using FACScan (Becton Dickinson). This analysis indicated that IL-17E-FC fusion protein bound to GM3104A cells. This binding was inhibited by IL-17RB-2 and IL-17RB-3 (isoforms of the present invention) but not IL-17 R.

EXAMPLE 10

IL-17E Polypeptide Induces Expression of Proinflammatory Cytokines

Since IL-17E polypeptide has been shown to bind to the IL-17 receptors of the present invention (SEQ ID NO: 2 and 5), effects of IL-17E polypeptide on the expression of pro-inflammatory cytokines can be correlated with activation of the IL-17 receptors of the present invention.

The conditioned media from 293E cells expressing either IL-17E-Fc fusion protein, IL-17B-Fc, IL-17C-Fc or IL-17D-Fc, was collected to use as ligand in the assay. Conditioned media containing IL-17B-Fc, IL-17C-Fc, IL-17D-Fc, and IL-17E-Fc were then concentrated (15x) using a 3Kd cut-off Centracon (Amicon, #4032), and reconstituted to 1x medium by adding fresh 20% FBS/1640 media.

Human B-lymphoblast cells (GM3104A, 1×10^6 cell/sample) were cultured in reconstituted concentrated condition media which contained each IL-17 ligand (IL-17E-Fc polypeptide, IL-

17B-Fc, IL-17C-Fc, IL-17D-Fc and human Fc). After incubation for 18 hours at 37°C and 5% CO₂, the media were collected and the amount of IL-1α, IL-1β, IL-6, IFN-γ, G-CSF, and TNF-α released into the media was measured with the appropriate Quantikine Immunoassay kit (R&D Systems) following the manufacturer's instructions. The results are summarized in table III. IL-17E-FC fusion protein induced the release of TNF-α, IL-1α, and IL-6 to a much greater extent than the other IL-17 ligands tested. Induction of IL-1β, IFN-γ, and G-CSF was not detected for any of the ligands.

Table III			
Ligand	TNF-α (pg/ml)	IL-1α (pg/ml)	IL-6 (pg/ml)
Mock CM	190	6	157.6
Human Fc	210	8	199
IL-17B	180	11	138
IL-17C	170	8	152
IL-17D	180	22	155
IL-17 like	460	25	362

EXAMPLE 9

IL-17E Overexpressing Transgenic Mice

As described in Example 7, IL-17E polypeptide is a ligand for the IL-17 like receptors of the present invention. The following examples describe transgenic mice which are overexpressing IL-17E polypeptide. The information from these mice is useful in determining the biological effects of activation and/or overexpression of the IL-17 receptors of the present invention.

A. Transgene Preparation.

The coding region of human IL-17E cDNA (SEQ ID NO: 22) with an altered Kozak sequence, CCACC, immediately upstream of the initiating ATG, was ligated into a liver-specific expression vector. The expression vector consisted of a 774-bp DNA fragment containing the hepatocyte control region (HCR) from the human apolipoprotein (apo) C-I/C-I' intergenic region on chromosome 19 (Simonet et al., *J. Biol. Chem.*, 268:8221-8229, 1993). The vector also contained a 1450-bp continuous piece of DNA which consisted of the human apoE gene 5'-flanking sequence, the first exon, the first intron and a portion of the second exon of the apoE gene (Simonet et al., *J. Clin. Invest.*, 94:1310-1319, 1994). An SV40 polyadenylation signal was located downstream of the cDNA insert sites. The integrity of the cDNA was verified by sequencing using standard methods known in the art.

B. Preparation and Analysis of IL-17E Overexpressing Transgenic Mice.

The resulting plasmid (denoted herein as ApoE-hIL-17) was purified and the transgene insert was isolated for microinjection. Single-cell embryos from BDF1 x BDF1-bred mice were injected essentially as described in Brinster et al. (*Proc. Natl. Acad. Sci. USA*, 82:4438-4442, 1985). Embryos were cultured overnight in a 37°C and 5% CO₂ incubator. Subsequently, 15 to 20 2-cell embryos were transferred to the oviducts of thirteen pseudopregnant CD1 female mice. Transgenic offsprings were identified by PCR screening with primers that amplify a 368-bp fragment of the human apoE first intron from DNA prepared from ear biopsies as described in Simonet et al. (*J. Clin. Invest.*, 94:1310-1319, 1994).

EXAMPLE 11

Necropsy Analysis of IL-17E Overexpressing Transgenic Mice.

At 8-10 weeks of age, 10 IL-17E overexpressing transgenic mice and five non-transgenic littermates were necropsied. Liver samples from the mice were flash frozen in liquid nitrogen at the time of necropsy. RNAs were isolated from each sample using the Perfect RNA Kit (Eppendorf) according to the manufacturer's instructions and analyzed by Northern blot analysis.

The Northern blot was generated by running 10 µg of RNA diluted in 1x RNA Loading Dye (Sigma) on a 1% formaldehyde-agarose gel. The gel was denatured in 50 mM NaOH and 150 and 55 mM NaCl. Subsequently, the gel was neutralized in 0.1 M Tris-HCl (pH 7.0) and 150 mM NaCl and blotted onto a Duralon membrane according to the manufacturer's instructions (Stratagene). The Northern blot was probed with a ³²P-labeled human IL-17E cDNA generated by the Rediprime System (Amersham). Hybridization was carried out in Express Hyb Solution and then washed according to the manufacturer's instructions. The hybridized blot was exposed to X-ray film (Kodak) for 72 hours at -80°C and then developed.

The Northern blot analysis indicated that the transgenic founder mice had increased expression of the IL-17E RNA as compared with the non-transgenic littermates. Of the 10 mice tested, those denoted as nos. 29, 52, 55, 61 and 66 had the highest level of IL-17E RNA expression. (See figure 8)

B. Expression Analysis on the Remaining Founders

Livers from the remaining transgenic founder mice along with control mice, were obtained by partial hepatectomy. The mice were anesthetized by isoflourane and a small transverse incision below the xyphoid process on the sternum was made to expose the liver. A suture was placed around the lobe of liver selected for excision at the point of

attachment. The lobe of liver was ligated and removed by cutting below the ligature and flash frozen in liquid nitrogen. The mouse was then checked for bleeding and the skin incision was closed with 1-2 autoclips (skin staples).
5 RNA was isolation from the liver and Northern blot analysis was carried out as described above. The hybridized blot was exposed to X-ray film (Kodak) for 24 hours at -80°C and then developed.

Northern blot analysis on the remaining founders
10 indicated that these mice expressed higher levels of IL-17E RNA in the liver as compared with non-transgenic littermates.

The mice denoted as nos. 11, 30, 33, 46 and 68 expressed the highest levels of IL-17 RNA. (See figure 9).

EXAMPLE 12

Pathological Analysis of IL-17E Overexpressing Transgenic Mice

A. Necropsy

20 In this study, seven, 6-8 week old, IL-17E overexpressing mice as well as five, 6-8 week old, non-transgenic littermates (two males and three females) were pathologically analyzed for a potential IL-17E phenotype. Mice nos. 29, 52, 61 and 66 were strongly positive for hepatic expression of IL-17E mRNA, while
25 mice nos. 1, 16 and 55 were weakly positive. The five non-transgenic control mice (nos. 2, 17, 28, 53 and 65) were negative. At necropsy, mice were weighed, blood was drawn for hematology and serum chemistries, and liver, spleen, kidney, heart, and thymus were weighed. Sections of liver, spleen,
30 lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal

vesicle, skeletal muscle, bone, and bone marrow were harvested for histologic analysis.

B. Histology

5 Sections of liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, skeletal
10 muscle, bone, and bone marrow from the IL-17E transgenic and non-transgenic mice were fixed overnight in 10% neutral buffered zinc formalin (Anatech, Battle Creek, Michigan), paraffin embedded, sectioned at 3 μ m, and stained with hematoxylin and eosin (H&E) for routine histologic
15 examination.

C. Immunohistochemistry

Immunohistochemical staining was performed on 4 μ m thick paraffin embedded sections using an automated DPC Mark 5
20 Histochemical Staining System (Diagnostic Products Corp, Randolph, NJ). Deparaffinized tissue sections were blocked with CAS BLOCK (Zymed Laboratories, San Francisco, CA), incubated with a rat anti-mouse monoclonal antibody directed against macrophages (F4/80, Serotec Inc., Raleigh, NC) or a
25 rat anti-mouse CD45R/B220 monoclonal antibody directed against all types of B cells (PharMingen, San Diego, CA). The primary antibody was detected using a biotinylated rabbit anti-rat immunoglobulin secondary antibody (Vector Laboratories, Burlingame, CA). Sections were then quenched with 3% hydrogen
30 peroxide and reacted with an avidin-biotin complex tertiary (Vector Laboratories). The staining reaction was visualized with diaminobenzidine (DAB, Dako Carpinteria, CA) and sections were counterstained with hematoxylin.

D. Gross Pathology Findings

Mesenteric lymph nodes from the four high expressing IL-17E transgenic mice (nos. 29, 52, 61 and 66) plus one of the low expressing mice (no. 55) were markedly increased in size. Similarly, the spleens from these five IL-17E transgenic mice were enlarged and exhibited a significant increase in weight (1.08 ± 0.27 SD % of body weight vs. 0.37 ± 0.12 SD % of body weight in non-transgenic control mice, $p=0.0007$). Mesenteric lymph nodes and spleens from two other low expressing transgenic mice (nos. 1 and 16) appeared normal. The raw organ weight data is shown in Table IV and significant differences are summarized in Table VI.

Table IV Raw Organ Weights for IL-17E Transgenic Mice vs. Non-Transgenic Mice

Group	Sex	TBW	Liver	%BW	Spln	%BW	Heart	%BW	Kidneys	%BW	Thymus	%BW
Non-Transgenic												
2	F	21.8	0.923	4.23	0.070	0.32	0.121	0.56	0.351	1.61	0.061	0.28
17	F	20.5	0.912	4.45	0.089	0.43	0.112	0.55	0.273	1.33	0.048	0.23
28	F	22.5	1.125	5	0.123	0.55	0.127	0.56	0.398	1.77	0.058	0.26
53	M	25.8	1.315	5.1	0.076	0.29	0.140	0.54	0.423	1.64	0.031	0.12
65	M	29	1.45	5	0.082	0.28	0.169	0.58	0.523	1.8	0.055	0.19
Mean				4.76		0.37		0.56		1.63		0.22
St. Dev.				0.39		0.12		0.01		0.19		0.06
IL-17E Transgenic												
1	F	31.9	1.406	4.41	0.118	0.37	0.151	0.47	0.433	1.36	0.071	0.22
16	F	22.5	1.121	4.98	0.085	0.38	0.115	0.51	0.350	1.56	0.061	0.27
29	F	24.4	1.439	5.90	0.333	1.36	0.123	0.5	0.861	3.53	0.061	0.25
52	M	25.6	1.583	6.18	0.223	0.87	0.129	0.5	0.356	1.39	0.074	0.29
55	F	19.1	1.181	6.18	0.196	1.03	0.122	0.64	0.388	2.03	0.04	0.21
61	F	24.5	1.401	5.72	0.190	0.78	0.118	0.48	0.372	1.52	0.059	0.24
66	M	25	1.47	5.88	0.338	1.35	0.162	0.65	0.433	1.73	0.026	0.1
Mean				5.61		0.88		0.54		1.87		0.23
St. Dev.				0.67		0.41		0.08		0.76		0.06

E. Hematology Findings

Four of the five IL-17E transgenic mice with enlarged mesenteric lymph nodes and spleens (the blood from mice nos. 29, 52, 55, 61 and 66 clotted and could not be evaluated) had moderate to marked increases in total leukocytes, neutrophils, lymphocytes, eosinophils, and large unstained cells (possibly large granular lymphocytes). The mean total leukocyte count for these four IL-17E transgenic mice was 11.93×10^3 ($\pm 4.47 \times 10^3$ SD) while non-transgenic control mice had a mean total leukocyte count of 3.09×10^3 ($\pm 0.79 \times 10^3$ SD, $p=0.003$). The mean neutrophil count in these four IL-17E transgenic mice was 2.29×10^3 ($\pm 0.67 \times 10^3$ SD) vs. 0.92×10^3 ($\pm 0.53 \times 10^3$ SD) in non-transgenic control mice, $p=0.032$. These four IL-17E transgenic mice had a mean lymphocyte count of 6.76×10^3 ($\pm 2.32 \times 10^3$) vs. 1.99×10^3 ($\pm 0.38 \times 10^3$ SD) in non-transgenic control mice, $p=0.0025$, a mean eosinophil count of 1.35×10^3 ($\pm 0.96 \times 10^3$ SD) vs. 0.03×10^3 ($\pm 0.01 \times 10^3$ SD) in non-transgenic control mice, $p=0.017$, and a mean large unstained cell count of 1.41×10^3 ($\pm 1.11 \times 10^3$ SD) vs. 0.10×10^3 ($\pm 0.05 \times 10^3$ SD) in non-transgenic control mice, $p=0.031$. Two of the IL-17E transgenic mice (nos. 55 and 66) also had a mild anemia characterized by a slight decrease in red blood cell number, hemoglobin, and hematocrit as well as slightly elevated platelet counts. The raw hematology data is shown in Table 2 and significant differences are summarized in Table 3.

Table V Raw Hematology Data for IL-17E Transgenic Mice vs. Non-Transgenic Mice

Group	WBC	RBC	HGB	HCT	PLT	MPV	Neut	Lymph	Mono	Eos	Baso	LUC
Non-Transgenic												
2	2.52	9.39	13.9	48.9	1179	5.0	0.69	1.64	0.02	0.03	0.01	0.13
17	3.48	10.12	15.1	50.9	938	5.1	0.72	2.63	0.02	0.04	0.01	0.06
28	2.45	9.51	14.8	49.5	1013	5.7	0.37	2.00	0.02	0.01	0.01	0.05
53	2.70	10.67	16.1	55.9	1353	5.0	0.61	1.88	0.04	0.04	0.01	0.11
65	4.30	11.55	17.8	61.4	1362	4.5	2.20	1.81	0.11	0.02	0.01	0.16
Mean	3.09	10.25	15.5	53.3	1169	5.1	0.92	1.99	0.04	0.03	0.01	0.10
St. Dev.	0.79	0.89	1.5	5.3	193	0.4	0.73	0.38	0.04	0.01	0.00	0.05
IL-17E Transgenic												
1	2.80	10.80	16.3	56.8	1113	5.2	0.69	1.91	0.03	0.02	0.01	0.14
16	3.49	10.29	15.8	54.7	1134	4.8	1.30	2.01	0.05	0.04	0.01	0.07
29	No Sample											
52	13.32	8.81	12.5	45.8	977	6.3	3.25	6.61	0.17	2.12	0.04	1.13
55	16.89	7.89	12.0	36.6	2758	5.4	1.84	9.80	0.09	2.14	0.04	2.99
61	11.32	9.18	14.1	50.0	1102	5.2	2.66	6.47	0.08	0.96	0.03	1.12
66	6.19	6.24	7.8	31.7	2195	4.4	1.42	4.16	0.05	0.16	0.01	0.40
Mean	9.00	8.87	13.1	45.9	1547	5.2	1.86	5.16	0.08	0.91	0.02	0.98
SD	5.71	1.66	3.1	10.0	744	0.6	0.94	3.06	0.05	1.01	0.02	1.09

Table VI Summary Data for Significant Differences in Organ Weights and CBC Values between IL-17E Transgenic Mice and Non-Transgenic mice

	HEAGP Transgenic Mice (n=4 or 5*)	Non- Transgenic Mice (n=5)	p value (t Test)
Spleen Weight as % Body Weight	1.08 \pm 0.27 SD*	0.37 \pm 0.12 SD	0.0007
Total Leukocytes (WBCs)	11.93 $\times 10^3$ \pm 4.47 $\times 10^3$ SD	3.09 $\times 10^3$ \pm 0.79 $\times 10^3$ SD	0.003
Neutrophils	2.29 $\times 10^3$ \pm 0.67 $\times 10^3$ SD	0.92 $\times 10^3$ \pm 0.53 $\times 10^3$ SD	0.032
Lymphocytes	6.76 $\times 10^3$ \pm 2.32 $\times 10^3$ vs SD	1.99 $\times 10^3$ \pm 0.38 $\times 10^3$ SD	0.0025
Eosinophils	1.99 $\times 10^3$ \pm 0.38 $\times 10^3$ SD	0.03 $\times 10^3$ \pm 0.01 $\times 10^3$ SD	0.017
Large Unstained Cells (LUC - Possibly Large Granular Lymphocytes)	1.41 $\times 10^3$ \pm 1.11 $\times 10^3$ SD	0.10 $\times 10^3$ \pm 0.05 $\times 10^3$ SD	0.031

5

F. Histopathologic Findings

Hematoxylin and eosin stained sections of liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary

10

gland, urinary bladder, ovary or testis, uterus or seminal vesicle, skeletal muscle, bone, and bone marrow were examined from seven IL-17E transgenic mice and five non-transgenic control littermates. B220 (specific for all B cells) and F4/80 (specific for macrophages) immunostained sections of lymph node and spleen were also examined from all mice. Five of the IL-17E transgenic mice (nos. 29, 52, 55, 61 and 66) had similar histologic findings characterized by marked mesenteric lymphadenopathy, splenic lymphoid hyperplasia and red pulp eosinophilic myeloid hyperplasia, and bone marrow eosinophilic hyperplasia. The most striking histologic finding was the mesenteric lymphadenopathy, which was characterized by massive nodal enlargement with loss of normal nodal architecture and medullary expansion by a mixed population of inflammatory cells containing a large number of eosinophils, reactive B cells (stained with B220) and plasma cells, macrophages (stained with F4/80) and multinucleated inflammatory giant cells (See figure 10). These five IL-17E transgenic mice also exhibited marked bone marrow eosinophilic myeloid hyperplasia (figure 11B) as well as moderate to marked splenic B cell lymphoid hyperplasia and red pulp eosinophilic myeloid hyperplasia (figure 11F). In addition, one of the IL-17E transgenic mice (no.29) also exhibited marked, chronic eosinophilic and suppurative pyelonephritis with renal pelvic dilation in one kidney and moderate chronic eosinophilic and suppurative pyelitis in the other kidney (figure 11J), while another IL-17E transgenic mouse (no. 55) exhibited severe, chronic eosinophilic and suppurative urinary cystitis with mild bilateral chronic eosinophilic and suppurative pyelitis. Lastly, four of the IL-17E transgenic mice (nos. 29, 55, 61 and 66) exhibited minimal to mild eosinophilic and lymphoplasmacytic colitis and/or ileitis.

G. Summary of Phenotypic Findings in Transgenic Mice Overexpressing Human IL-17E Polypeptide

Five of the IL-17E overexpressing transgenic mice (nos. 29, 52, 55, 61 and 66) had a similar phenotype, characterized by a leukocytosis with marked elevations in eosinophils, lymphocytes, and large unstained cells which may be large granular lymphocytes, a marked lymphadenopathy with a marked eosinophilic component, bone marrow eosinophilic myeloid hyperplasia, and splenic B cell lymphoid hyperplasia and eosinophilic myeloid hyperplasia. Two of the IL-17E transgenic mice (nos. 55 and 66) also exhibited mild anemia and thrombocytosis. In addition, IL-17E transgenic mice nos. 55 and 29, exhibited eosinophilic and superlative inflammation of their kidneys and/or urinary bladder. Lastly, four of the IL-17E overexpressing transgenic mice (nos. 29, 55, 61 and 66) had minimal to mild eosinophilic and lymphoplasmacytic colitis and/or ileitis. All of these findings suggest that the IL-17E polypeptide plays a role in inflammation and myelopoiesis, particularly in the development, stimulation, and/or recruitment of eosinophils and B lymphocytes, and that the IL-17 receptor like polypeptides of the present invention, which bind to IL-17E, mediate this inflammation and myelopoiesis.

EXAMPLE 13

Transgenic Phenotype of IL-17E Overexpressing Mice

Phenotype analysis was performed on 10 transgenic mice and 5 non-transgenic littermates. A femur, peripheral blood (obtained by cardiac puncture) and a longitudinal half section of spleen were obtained from each transgenic mouse and their littermate control. Five of the transgenic mice analyzed (nos. 29, 52, 55, 61 and 66) exhibited phenotypic changes.

To analyze the phenotype of the transgenic mice, the major hematopoietic populations including activated T cells were quantitated. In addition, the tissue and lineage

specific expression of IL-17 receptor like polypeptides of the present invention (IL-17RB), was quantitated as described in Antonysony et al. (*J. Immunol.*, 162: 577-584, 1999) and as also described in Example 7 herein.

5 The following antibody panel was designed to make the above-identified measurements with fluorescent activated cell sorting (FACS). Helper T cells were detected with the antibody CD4-PE and were compared to an early activation marker detected by the antibody CD69-FITC. A pan T cell
10 marker detected with antibody CD3-FITC was compared to killer T cells detected with the antibody CD8-PE. Monocyte lineage cells detected with the antibody CD14-FITC was compared to B lineage cells (preB to mature surface immunoglobulin positive B cell) marker detected with the antibody CD19-PE.
15 Granulocytes detected with the antibody GR-1-FITC were compared to natural killer cells detected with the antibody NK1.1-PE. The expression pattern of the IL-17 receptor like polypeptides (IL17RB), detected by binding of recombinant IL-17E-Fc fusion protein, was compared to B cells detected with
20 the antibody CD45R-PE, to Helper T cells detected with the antibody CD4-PE, and to dendritic cells detected with the antibody CD11C-PE.

The transgenic mice and non-transgenic littermates were sacrificed and the femurs and spleens were dissected.
25 Cell suspension from the femoral bone marrow and the spleen were made, washed twice and resuspended in PBS/0.5% BSA. The cell number of each cell suspension was quantitated with a Coulter Z1 Coulter Counter using a 100 μ m aperture and a lower threshold setting of 4 μ m. A 10 μ l aliquot of each cell
30 suspension was added to 10 ml of Isoton buffer containing 3 drops of Zapoglobin (to lyse the red blood cells) and counted. The cell suspensions were incubated with Fc-block (CD 16/32) for 15 minutes at 4°C. Subsequently, 1×10^6 cells (suspended in

PBS/0.5% BSA) were added to each antibody-containing well on a 96 well plate.

In addition, peripheral blood samples from the transgenic mice and non-transgenic littermates were obtained by cardiac puncture and CBC analysis was performed. Subsequently, the remaining blood was divided equally among 8 wells containing the antibodies on a 96 well plate.

The cell suspensions and blood samples were incubated in the presence of the antibodies for 30 minutes at room temperature. Subsequently, the cells were washed twice and lysed with FACS lysing buffer (200 µl/well; Becton Dickinson) for 15 minutes at room temperature in order to eliminate the red blood cells. After lysing, the cells were washed and resuspended in 400 µl of FACS buffer and analyzed by flow cytometry.

In the 5 transgenic mice which exhibited a phenotype (nos. 29, 52, 55, 61 and 66), there was a striking increase in CD19+ cells (B cells) in the peripheral blood. As shown in figure 12, the absolute number of CD19+ cells was increased up to 5 fold compared to controls. In addition, there was a 2-4 fold increase in absolute number of CD19+ cells in the spleen as shown in figure 13. In the femoral bone marrow, there was a slight decrease in CD19+ cells (figure 14). Staining for CD45r followed a similar trend. The peripheral blood and spleens isolated from the transgenic mice also exhibited a 2-3 fold increase in the absolute number of helper T cells (CD4+ T lymphocytes). (See figures 15 and 16; respectively)

The transgenic mice had a consistent appearance of a large population of cells (e.g., 33% granulocytes) bearing light scatter properties similar to those of eosinophils (figures 17 and 18). In addition, the cells do not express the granulocytic marker. There was also a consistent appearance of a smaller but distinct population of granulocyte

like cells (e.g., 8-17% of granulocytes) that express the IL-17 receptor like polypeptides in blood and bone marrow. (See figures 19 and 20). Based on correlations with scatter plots, the transgenic mice seem to have the following multi-lineage phenotype: CD4+, CD45R+, CD11c+, and are large and granular.

This analysis indicated that within the transgenic mice there was a clear emergence of an eosinophil-like population in the femoral bone marrow and peripheral blood. As shown in figure 21, the scatter profile of these cells closely resembles a "text-book" example of the forward vs. side scatter (size vs. granularity) properties of eosinophils.

There was also an important increase in the absolute number (and compartmental percentage) of circulating and splenic CD19+ B cells. Although the CD19+ lymphocytes were not positive for the activation marker CD69+, their increase in absolute number in the periphery and slight decrease in the bone marrow is suggestive of migration to peripheral tissues where proliferation is taking place.

The appearance of a multi-lineage phenotype in blood and bone marrow is suggestive of a lymphoma like phenotype. Furthermore, since the IL-17 receptor like polypeptides (SEQ ID NOS: 2 and 5) seems to be upregulated on these cells, it is suggestive that this population may be reactive to the omnipresence of IL-17 like protein. Together with the fact that there is clear eosinophilia in these mice, the multi-lineage phenotype closely fits the description of an acute myelomonocytic leukemia (M4 AML) (Campena & Behm, *J. Immunol. Meth.* 234,:59-75, 2000).

EXAMPLE 14

Northern Blot Analysis of IL-17 Receptor Like RNA

Northern blot analysis was carried out to determine which cell lines expressed the IL-17 receptor like RNA. Total RNA was isolated from 17 cell lines using the RNeasy Mini Kit (cat. No. 74104, Qiagen, Valencia, CA). The probe was generated by PCR using the following primers (primer 2445-34: CATTTTCCTACATCGGCTTCCCTG; SEQ ID NO: 26; and primer 2429-61 TGAATCTGGCTTCTTTCACTGC; SEQ ID NO: 27). The probe was labeled with ³²P-dCTP (cat. No. AA0005; Amersham Pharmacia Biotech) using Rediprime II Kit (cat. No. RPN 1633; Amersham Pharmacia Biotech). The Northern Blot analysis was performed with the Northern Max-Gly Kit (cat no. 1946, Ambion).

The following human, mouse and rabbit cell lines were tested and the level of expression of IL-17 receptor like RNA is indicated.

	Human Cell Lines:	Level of Expression
20	GM3104A (B-lymphoblast cell)	+++
	CCRF-SB (B-lymphoblast cell)	+++
	CESS (Lymphoma)	+
	THP-1 (Acute monocytic leukemia)	+/-
	DAMI (Megakaryocytes)	+/-
25	H-9 (T-cell lymphoma)	-
	CCRF CEM (T-lymphoblast cell)	-
	MOLT 4 (T-cell lymphocytes)	-
	Hs 67(Thymus, normal)	-
	Jurkat E6-1 (T-cell leukemia)	-
30	J 45.01 (T-cell leukemia)	-
	BW5147.3 (T-cell lymphoma)	-
	CCRF HSB2 (T-lymphoblast cell)	-
	AML 193(s) (Acute monocytic leukemia)	-
35	Animal cell lines:	
	HIG - 82 (Rabbit synoviocyte)	-
	C 1498 (Mouse lymphoma)	-
	A 20 (Mouse B cell lymphoma)	-